Available online at www.sciencedirect.com
SCIENCE DIRECT®
Scientia Horticulturae 107 (2005) 97–102
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Short communication

A modified green-grafting technique for large-scale virus indexing of grapevine (*Vitis vinifera* L.)

Ranjith Pathirana *, Marian J. McKenzie

New Zealand Institute for Crop and Food Research Limited, Private Bag 11600, Palmerston North, New Zealand

Received 24 December 2004; received in revised form 19 May 2005; accepted 9 June 2005

Abstract

A modified method of green-grafting was investigated for use in viral indexing of grapevine stock. *Cabernet franc* and *Cabernet sauvignon* scions infected with grapevine leafroll-associated virus 3 (GLRaV3) were cleft-grafted on to virus-free indicator rootstocks LN33 and *C. sauvignon*, and were maintained in polystyrene containers containing vermiculite soaked with Huglin and Julliard solution. Using this method plants could be transferred to soil 3–4 weeks following grafting. Leafroll symptoms: reddening of leaves, leaf rolling, and interveinal discolouration, were scored at 3–4 weeks and again at 12 weeks. Double antibody sandwich–enzyme linked immunosorbent assay (DAS–ELISA) was used to validate the visual symptoms of disease. At both times it was clear that *C. sauvignon* was a more sensitive indicator of GLRaV3 than LN33, with 80% of grafts showing symptoms at 3–4 weeks and 90% showing symptoms at 12 weeks. The results indicate that the technique described here is effective and suitable for large-scale indexing of grapevine stock for viral contamination.

Keywords: Closterovirus; ELISA; Leafroll virus; Grapevine; Green-grafting; Nutrient solution

1. Introduction

Wine exports from Australasia have substantially increased in the past decade due to the expansion of land area supporting grapevines, the diversification of grape varieties being cultivated and increased worldwide demand. This has increased the risk of previously unknown viruses being introduced into vineyards. Because the means to reduce losses caused by viral infection once a vineyard is established are limited (Martelli et al., 1993) all materials used must be free from viruses. To maintain the growth of the wine industry, particularly in the Southern Hemisphere, it is vital that robust, sensitive, rapid and reliable techniques for assessing grape clones for viral pathogens are developed.

Currently the New Zealand Ministry of Agriculture and Forestry (MAF) requires that imported grapevine stock undergo quarantine for a minimum period of 16 months while being inspected for pathogens. Methods used during inspection include graft indexing of woody cuttings in which the incoming stock is grafted on to indicator rootstocks. Although reliable, this method is time consuming and labour-intensive, often extending the quarantine period to 3 years. On the other hand, rapid and reliable serological (Kearns and Mossop, 1984; Garnsey and Cambra, 1993; Monis and Bestwick, 1996) and molecular (Wan Chow Wah and Symons, 1997; Goszczynski and Jooste, 2003) techniques are limited to known viruses for which antibodies and genome sequences are available, moreover these methods are costly. Therefore, it is desirable to test other methods of diagnosing viral diseases in grapevines and apply those that are cost-effective and rapid.

A relatively new technique, green-grafting, has been developed for the vegetative propagation of grapevines. It has been successfully adopted in several grape-growing countries to rapidly diagnose viral disease (Walter et al., 1990; Lahogue et al., 1995; Kassemeyer et al., 1997; Kaserer et al., 2003). Unfortunately this method has not been adopted in Australasia (Nicholas, 2001; Golino, 2002; MAF, 2004) due to the lack of reliable research data on the method for this region.

* Corresponding author. Tel.: +64 6 356 8300; fax: +64 6 351 7050.
E-mail address: PathiranaR@crop.cri.nz (R. Pathirana).

0304-4238/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2005.06.002

Here we report the use of a modified green-grafting method in which polystyrene containers containing vermiculite soaked with a nutrient solution were used to establish large numbers of grafted shoots in a limited space. The method was assessed to determine the minimum period required to index GLRaV3 infection using two indicator rootstocks.

2. Materials and methods

2.1. Plant material

Grapevine (*Vitis vinifera* L.) material was provided by the Marlborough Foundation Vineyard of New Zealand Grapevine Improvement Group (NZGVIG). Woody cuttings of *Cabernet sauvignon* and *Cabernet franc* certified as infected solely with GLRaV3 and two virus-free varieties, *C. sauvignon* and LN33, were obtained. The woody cuttings were trimmed to lengths with one bud each and planted bud-side up in standard potting mix under a mist bed. Cuttings that produced shoots by 3–6 weeks were removed and planted in 5 L polythene bags in potting mix.

Additional green shoots were forced from herbaceous cuttings (20 cm long) on rockwool cubes (Grodan Rockwool size 25/40, Veg-Gro Supplies, Auckland, New Zealand) and watered twice weekly with one of three nutrient solutions (Table 1). The shoots were maintained under saturated humidity.

2.2. Green-grafting

Shoots chosen for grafting had a diameter of 5–8 mm, and were at 3–6 nodes below the active bud. Their leaves were trimmed to about half their original size before grafting. Three methods of green-grafting were assessed: manual cleft-, machine cleft- and manual splice-grafting. In cleft-grafting the rootstock and scion cuttings, having one bud each, were cleft-grafted either by hand, or using a grafting machine (Field Craft Top Grafter, Riggett Industries, Gisborne, New Zealand). A slit was made down the centre of the rootstock and a long tapered wedge was made on the scion. The rootstock and the scion had the same diameter at the point of contact so that the cambium layers of the union matched (Martelli et al., 1993). The assembled graft (Fig. 1a) was wrapped with commercial grafting tape and supported by a plastic clothes peg (Fig. 1b). The base of the rootstock was then fitted into a hole in the lid of a 150 ml polystyrene container (Biolab, Auckland, New Zealand) filled with vermiculite soaked in Huglin and Julliard (1964 as quoted by Martelli et al., 1993) nutrient solution (Table 1, Fig. 1b) and maintained at 25 °C under saturated humidity for 3–4 weeks.

In splice-grafting sloping cuts 2–3 cm long were made by hand on the scion and the rootstock. Again the scion and the rootstock had one bud each and the graft was made and maintained in a polystyrene container under saturated humidity as in the previous method.

After 3–4 weeks in the humidity chamber the lid of the polystyrene container was carefully opened and the rooted graft transferred to soil in nursery trays, along with the lid. At this stage the grafts had produced many roots (Fig. 1c) and were easily acclimatised to greenhouse conditions.

2.3. Experimental design, data recording and analysis

Two virus-free rootstocks (*C. sauvignon* and LN33) and two virus-infected scions (*C. franc* and *C. sauvignon*) were used, making a total of four graft combinations. The green shoots to be used as rootstocks and scions for green-grafting

Table 1
Composition of nutrient solutions used to induce green shoots for grafting experiments

Chemicals	Knop medium	Modified Hoagland[a]	Huglin and Julliard[b]
$Ca(NO_3)_2{\cdot}H_2O$ (mM)	3	5	
KNO_3 (mM)	1.5	5	7.912
$MgSO_4$ (mM)	1.25	2	1.217
KH_2PO_4 (mM)	1	1.3	
$(NH_4)_2{\cdot}HPO_4$ (mM)			1.514
Fe-EDTA Sodium salt (mM)	0.04	0.1	0.1
H_3PO_4 (mM)			0.173
H_2SO_4 (mM)			0.51
$MnCl_2{\cdot}2H_2O$ (μM)		9.15	
$MnSO_4{\cdot}H_2O$ (μM)			8.88
$ZnSO_4{\cdot}7H_2O$ (μM)		0.77	5.217
H_3BO_3 (μM)		46.25	24.26
$CuSO_4{\cdot}5H_2O$ (μM)		0.32	2.0
$H_2MoO_4{\cdot}H_2O$ (μM)		0.37	
$(NH_4)_6{\cdot}Mo_7O_{24}{\cdot}4H_2O$ (μM)			.04

pH in all media were adjusted to 6.

[a] Modified from Hoagland and Arnon (1938).

[b] Huglin and Julliard (1964 as quoted by Martelli et al., 1993).