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The sustainability of old grapevine mother plants in relation to new mandatory diagnostic tests for virus control

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Abstract: In 2011, the methods to perform phytosanitary tests to check for viruses in grapevine nurseries were reassessed and new regulations were defined (DM 13 December 2011). The mandatory tests require serological assays for the diagnosis of five viruses in grapevine mother plants transplanted in Tuscany in 2001 or before. The aim of the present paper is to report the impact of certification programs applied before 2001 in Tuscany and the sustainability of older mother plants with relation to the new mandatory diagnostic tests. Among the cultivars, virus infection was reported in 19.2% of pool samples, whereas 2.4% of rootstock pool samples showed a compromised health status. GLRaV-3 is the most frequently found virus (10.4% and 1.3% of cultivar and rootstock pools, respectively), and it is also included in the most frequent multiple infections. Multiple infections represent about 25% of infected cultivar pools and almost 50% of infected rootstock pools.

1. Introduction

Italian production of propagated certified grapevines by nurseries is regulated by laws (DM 8 February 2005; DM 7 July 2006) that define the procedures to obtain a certification for propagative material in order to handle healthy plants. These regulations provide detailed guidance for the registration of the primary source to be maintained by the conservative breeder and for the production of basic material or mother plants, the latter grown in nurseries and used to produce certified materials delivered to the growers. In 2009, the "Working group ARNADIA - grapevine viruses" was established, within the Italian Ministry of Agriculture Finalized Project "ARNADIA", with the purpose of producing validated reference diagnostic protocols for the control and monitoring of plant pathogens of phytosanitary interest. In 2011, the methods proposed to perform phytosanitary testing were reassessed (DM 13 December 2011) and it was established that mother plants grown in Italian nurseries (cultivars or rootstocks) have to be checked for virus infections 10 years after transplanting. The first deadline was set for 30 June 2012 to consider mother plants transplanted in 2001 or earlier. Obviously, these mother plants were checked and produced according to older regulations (starting with DPR 24 December 1969) that define pathogens (absence of grapevine leafroll and fanleaf degeneration disease) and methods for their assay (biological indexing) differently compared to the most recent regulations. Presently, plants are tested for the following viruses: Grapevine leafroll associated virus -1 (GLRaV-1) and -3 (GLRaV-3), Grapevine fanleaf virus (GFLV), Arabis mosaic virus (ArMV) and Grapevine virus A (GVA). The significant presence of these viruses was recently reported in Tuscany after assays carried out in sanitary selection programs. From 1997 to 2004, health tests conducted on 172 uncertified plants selected from the Tuscan costal area (Elba Island, Lucca and Maremma) showed a critical phytovirologic condition, with 97.1% infected plants (Materazzi *et al*., 2006 a). In the period 2000-2004, health tests conducted on 318 uncertified grapevine plants selected in D.O.C. or D.O.C.G. areas of Tuscany (Chianti Classico, Montalcino and Montepulciano) revealed that 58.8% vines were virus-infected (Materazzi *et al*., 2006 b). These findings cannot be transferred to nurseries, considering their use of certified materials. In any case, the virologic status of uncertified grapevine in Tuscany indicates the presence of grapevine viruses and related vectors, underlining the importance of periodic verifications in grapevine nurseries to guarantee the highest health standards of plant production and to help reduce

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the spread of grapevine pathogens, as determined by the updated regulations.

In this paper, we report the results obtained from serological (ELISA) tests for the diagnosis of five viruses in grapevine mother plants (cultivars or rootstock) transplanted in 2001 or before, as dictated by DM 13 December 2011. The aim is to report the impact of certification programs applied before 2001 in Tuscany and the sustainability of older mother plants as stated by the new mandatory diagnostic tests.

2. Materials and Methods

Plant sampling and ELISA tests

Plant sampling and ELISA tests were carried out following procedures defined by the Working group ARNA-DIA - grapevine viruses, included in DM 13 December 2011. In accordance with legislation, the following steps were undertaken. Sampling was performed beginning in November 2011 in 33 nurseries, collecting sample pools composed by homogenous material from five plants. Phloem tissue (2 g) was collected from pools and mechanically ground (Tissue Lyzer with 10 ml-grinding jar, Qiagen, Venlo, Netherlands) with extraction buffer. ELISA test was performed using commercial polyclonal antibodies as well as negative and positive controls (Agritest, Bari, Italy). Absorbance at OD_{405} nm was recorded by photometry (Titertek multiskan, Titertek Instruments Inc., Huntsville, USA). Readings were normalized as R value (OD-treated explant/OD-HC), identifying the R= 2 threshold which distinguishes the positive versus the negative response (Monette, 1983).

3. Results

The ELISA test (Table 1) showed that 19.2% of cultivar pools were infected with at least one of the viruses. All five viruses were found in cultivar samples, but GLRaV-3 was considerably more frequent than the others, followed by GVA. The combination of these two viruses also represent the most frequent multiple infection. Multiple infections represent about 25% of infected pools and they are characterized by 13 different virus combinations. The least frequent virus was ArMV.

With regard to rootstock pools, there was a low rate of infection (2.3%), even if GLRaV-3 was still the most frequent virus detected. This virus was also included in the most frequent multiple infections that, for rootstocks, represent almost 50% of infected pools. Multiple infections were reported in ten different virus combinations. Also in rootstocks, ArMV was the least frequent virus.

4. Discussion and Conclusions

Monitoring revealed GLRaV-3 as the most frequent virus in cultivar or rootstock mother plants, as reported in sanitary selection research previously carried out in Tuscany (Triolo and Materazzi, 2004; Materazzi *et al*., 2006 a) and other Italian areas (Digiaro *et al*., 2000; Bica *et al*., 2002; Martelli, 2002). Similarly, the low frequency of ArMV is in agreement with other health checks performed in Tuscany on uncertified plants (Borgo *et al*., 2000; Materazzi *et al*., 2006 a). Viruses were frequently detected in multiple infections, in particular in rootstocks, with a wide range of combinations.

Table 1 - Rates of virus infection for cultivar or rootstock pools detected by ELISA test

Mother plant	No of checked pool		No of infected pool	% of virus infection	
Cultivar	712		137	19.2	
Rootstock	1523		36	2.4	
Infected pool out of total (%)	GLRaV-1	GLRaV-3	GFLV	ArMV	GVA
Cultivar	2.2	10.4	1.8	1.4	5.1
Rootstock	0.9	1.3	0.9	0.6	0.7
	Pool infected by multiple viruses out of total (%)				
	Cultivar			Rootstock	
GLRaV-3/GVA	2.11		GLRaV-1/GLRaV-3	0.33	
GLRaV-1/GLRaV-3/GVA	0.56		GLRaV-3/GVA	0.13	
GLRaV-1/GLRaV-3/GVA/GFLV	0.42		GLRaV-3/GFLV	0.13	
GLRaV-1/GLRaV-3	0.28		ArMV/GVA	0.13	
GLRaV-1/GFLV	0.28		Others	0.46	
GLRaV-3/GVA/ArMV	0.28				
Others	0.98				
Total multiple infections	4.91		Total multiple infections	1.18	

Considering that these findings represent the first application of DM 13 December 2011, it is not possible to evaluate the health status of mother plants grown in Tuscan nurseries. Moreover, comparison to other Italian areas is not relevant because no homogeneous data are available. In any case, these finding can be a starting point to evaluate the health trend of plants in Tuscan nurseries.

The current health status of mother plants may be due to re-infection events as all tested viruses are known to be vector-transmitted (Golino *et al*., 2002; Andret-Link *et al*., 2005; Zorloni *et al*., 2006; Demangeat *et al*., 2010; Tsai *et al*., 2010) and relative vectors have been found in Tuscany. However, the significant improvement in diagnostic tests over the last 30 years do not seem to exclude that the primary source or basic material were originally infected. Even if these categories are considered in DM 13 December 2011, there is no updated health information available that can reconstruct the propagation links. In this case, the application of traceability tools such as electronic identification (Bandinelli *et al*., 2009; Luvisi *et al*., 2012 a, b) could support retrieval of health information. Moreover, the activity of local conservative breeders, such as the Associazione Toscana Costitutori Viticoli (TOS.CO.VIT.) set up in Tuscany in 2003 (Triolo, 2011), can promote the use of certified plants selected according to the most recent regulations.

Considering that the rate of infected cultivars and rootstocks was found to be very low during verifications carried out during Tuscan sanitary selections, these findings confirm that the use of certified plants helps reduce the spread of grapevine viruses.

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