

SHORT NOTES

Detection of viruses in grapevines imported in Missouri from Eastern European countries

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Summary. A total of 123 cultivars or selections were imported from Eastern European countries for testing in Missouri, USA. All cultivars were tested by ELISA and grafted indicator grapevines for the presence of important virus diseases. The predominant virus disease was grapevine fleck virus (GFkV) with other virus diseases occurring at much lower rates.

Key words: viruses, grapevines, Eastern Europe.

In Missouri (USA), wine grape cultivation is a comparatively young and small industry. The total area planted with commercial vineyards is about 250 ha. The cultivars grown are predominantly French hybrids with some local varieties.

Since the Missouri grape and wine industry required an expansion of the available range of wine grape cultivars, an importation program began in 1991 at the request of the Missouri Grape and Wine Advisory Board, in response to the need expressed by local wineries. New wine grape cultivars were sought, initially from Hungary and the Czech Republic, then from Bulgaria, Romania, Ukraine, and Moldova, i.e. countries that had carried out grape breeding programs for the development of cultivars with resistance to fungal diseases and tolerance to low temperatures, combined with good technologi-

cal traits for wine making. As a result, a total of 123 varieties and accessions were imported, including some of the most recent hybrids developed by east-European grape breeders. Before release, all accessions were tested for the presence of viruses by biological indexing and ELISA, as reported hereafter.

Two 1.5-cm-long chips of cortical tissue from each donor vine were grafted on three rootlings each of the indicators *Vitis rupestris* St. George, LN-33, Kober 5BB and Cabernet Franc. The indicators were grown in a shade house for one month prior to transplanting to the field, where they were observed for symptom expression four times annually for two growing seasons.

All chip-budded indicators and imported accessions were also tested by ELISA. Cortical shavings from canes and leaf veins were crushed in 0.1 M Tris-HCl buffer, pH 8.2 (Tris 60.5 g, NaCl 8 g, PVP 40000 20 g, PEG-6000 10 g, NaN₃ 0.2 g, Tween 20 0.5 ml in 1000 ml distilled water). Commercial

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Table 1. Virus infected grape cultivars from eastern Europe countries.

Cultivars	Country	Viruses					
		GFLV	GFkV	GLRaV 1	GLRaV 3	GVA	GVB
I – 55/8	Bulgaria	- ^a	+ ^b	-	-	-	-
II – 70/21	Bulgaria	-	+	-	-	-	-
XI – 38/57	Bulgaria	-	+	-	-	-	-
Mavrud	Bulgaria	-	+	-	-	-	-
Red Misket	Bulgaria	-	+	-	-	-	-
Pamid	Bulgaria	-	+	-	-	-	-
Kristaly	Bulgaria	-	+	-	-	-	-
Medina 18-9	Bulgaria	-	+	-	-	-	-
Medina 18-4	Bulgaria	-	+	-	-	-	-
Medina 18-1	Bulgaria	-	+	-	-	-	-
Ivan	Hungary	-	-	-	+	+	-
Viktor	Hungary	-	+	-	+	-	-
SK 77-4/5	Hungary	-	+	-	-	-	-
CSFT 194	Hungary	-	+	-	-	-	-
RF-16	Hungary	-	+	-	-	-	-
K-37	Hungary	-	-	+	-	-	-
K-30	Hungary	-	-	+	-	-	-
Ir 18/1	Hungary	-	-	-	+	-	-
MI – 5-26	Czech Republic	-	+	-	-	-	-
MI 5-114	Czech Republic	-	+	-	-	-	-
L 4-9-18	Czech Republic	-	+	-	-	-	-
Purpuriu	Romania	-	-	-	-	+	-
Otilia	Romania	+	-	-	-	-	-
Tamaioasa Romaneasca	Romania	-	+	-	-	-	-
N 31-53-37	Ukraine	-	+	-	-	-	-
Muscat Odesskij	Ukraine	-	+	-	-	-	-
Ovidiopoljskij	Ukraine	-	+	-	-	-	-
Golubok	Ukraine	-	+	-	-	-	-
Rubin Tairovskij	Ukraine	-	+	-	-	-	-
Illitchovskij rannij	Ukraine	-	+	-	-	-	-
Guzal kara	Ukraine	-	+	-	-	-	-
Vostorg	Ukraine	-	+	-	-	-	-
40 let Oktjabrja	Ukraine	+	-	-	+	-	-
Irshai oliver	Ukraine	-	+	+	-	-	-
Sucholimanskij belij	Ukraine	-	+	-	-	-	-
Kishmish Ljunda	Ukraine	-	+	-	-	-	-
Kishmish belij togopskij	Ukraine	-	+	-	-	-	-
Furmint	Ukraine	+	-	-	-	-	-
Kokur belij	Ukraine	-	-	-	+	-	-
Riton	Moldova	-	+	-	-	+	-
Pitos	Moldova	-	-	-	-	+	-
Flacara	Moldova	-	+	-	-	+	-
Negru de jaloveni	Moldova	-	+	-	-	+	-
III 77-20	Moldova	-	+	-	-	-	-

^a -, negative results by ELISA and grafted grapevine indicator.

^b +, positive results by ELISA and grafted grapevine indicator.

ELISA kits by Agritest (Italy) to detect the following viruses were used: Grapevine fanleaf virus (GFLV), Grapevine fleck virus (GFkV), Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine leafroll-associated virus 1 (GLRaV-1) and Grapevine leafroll-associated virus 3 (GLRaV-3). Standard DAS-ELISA, DIAS-ELISA and DASI-ELISA procedures were used (Van Regenmortel, 1982). For GVA, plates were precoated with protein A and incubated for 2 h at 30°C. For GVA, GFLV, GLRaV-1, and GLRaV-3, coating antibodies and conjugate antibodies were incubated for 2 h at 37°C. For GFkV and GVB, the antibodies (polyclonal, monoclonal and conjugate) were each incubated for 2 h at 37°C. Plants extracts, including positive and negative controls, were incubated overnight at 4°C. Results were scored after the addition of para-nitrophenyl phosphate in 10% diethanolamine buffer pH 9.8. Substrate incubation times for GFLV, GFkV, GVB and GLRaV-3 were 30-60 min, for GVA and GLRaV-1 60-120 min. The results were determined by relating the absorbance at 405 nm of the samples to a healthy threshold value. Absorbance greater than twice the healthy value was considered positive for the viruses tested.

The results of biological indexing and serological (ELISA) testing were basically in agreement, although with some viruses, such as leafroll-associated closteroviruses and vitiviruses, ELISA gave a much faster response compared to the reaction of the indicator plants.

As shown in Table 1, which summarised the results of ELISA testing, GFkV was by far the most widespread virus being found in 79.5% of the samples. This very high incidence may reflect the fact that breeders in east-European countries do not screen *Vitis vinifera* cultivars for GFkV (Milkus, 1983), a virus which, according to Nel and Engelbrecht (1972) does not influence grape productivity, although it may have an effect at scion-rootstock interface causing incompatibility. The incidence of other viruses was: 6.8% for GFLV; 6.8% for GLRaV-1; 11.4% for GLRaV-3 and 13.6% for GVA. GVB was not detected.

By comparison with the sanitary status of grapevines grown in the Mediterranean and Eastern countries, as determined by recent surveys

(Martelli, 2000; Digiario *et al.*, 2000), grapevines imported from eastern Europe appeared to be in a better health condition, possibly because many of the accessions were newly obtained selections or hybrids. GFkV constituted a notable exception; its incidence, compared with that observed in many west-European and Mediterranean countries (Digiario *et al.*, 2000; Martelli *et al.*, 2000), was extremely high. It would seem, however, that in grape-growing regions like Missouri where own rooted hybrids are used, GFkV should not constitute a major problem. In any case, most of the imported GFkV-infected selections were successfully subjected to virus elimination by heat treatment and meristem shoot tip culture (Milkus *et al.*, 2000).

Acknowledgements

The authors wish to thank Prof. G.P. Martelli for general assistance and for the critical review of the manuscript, and both the Missouri Grape and Wine Advisory Board and the Plant Industry Division of the Missouri Department of Agriculture for financial support.

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Accepted for publication: June 5, 2000