Evaluation of sanitary status of grapevines in the Czech Republic

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ABSTRACT

A survey was made to evaluate sanitary status of grapevines in the Czech Republic with regard to occurrence of economically important viruses. Propagation material of 109 grapevine clones was tested for presence of *Grapevine fanleaf virus*, *Arabis mosaic virus*, Grapevine leafroll-associated virus 1, *Grapevine leafroll-associated virus 3*, *Grapevine virus A*, *Grapevine virus B* and Grapevine fleck virus. Dormant canes were collected and cortical scrappings were analyzed by DAS-ELISA. All seven viruses tested were found to be widely spread in Czech propagation material of grapevine. From 330 individual vines tested, 148 vines were found to be infected with at least one virus. From 109 clones tested, in 98 clones at least one vine negative for tested pathogens was found. Such vines were promoted as candidate plants into screenhouse in Faculty of Horticulture Lednice and will be further tested by other methods. Sanitation of infected grapevine clones is needed in near future.

Keywords: *Grapevine fanleaf virus*; *Arabis mosaic virus*; *Grapevine virus A*; *Grapevine virus B*; Grapevine fleck virus; Grapevine leafroll-associated virus 1; *Grapevine leafroll-associated virus 3*; certification; ELISA; propagation material

Planting of healthy plant material is a base for high quality yield. Healthy status is important especially in crops propagated vegetatively, where large number of viruses occurs. In such crops, certification of planting material under state control is needed. To start the certification of grapevine in the Czech Republic, survey on health status of clones of grapevine varieties and rootstocks was started.

The presence of seven economically important viruses was evaluated: two nepoviruses, *Grapevine fanleaf virus* (GFLV) (Auger et al. 1992, Walker et al. 1994) and *Arabis mosaic virus* (ArMV) (Ipach et al. 1992), two vitiviruses, *Grapevine virus A* (GVA) (Peressini et al. 1991, Digiaro et al. 1994) and *Grapevine virus B* (GVB) (Bonavia et al. 1996), two closteroviruses, Grapevine leafroll-associated virus 1 (GLRaV-1) (Fortusini et al. 1996) and *Grapevine leafroll-associated virus 3* (GLRaV-3) (Borgo 1990) and one unassigned virus, Grapevine fleck virus (GFkV) (Sabanadzovic et al. 1996).

MATERIAL AND METHODS

From grapevine clones, 109 clones of 40 varieties and 7 rootstocks, see Table 1, maintained at eight viticulture breeding stations, were selected for certification. From propagation material of each clone, several vines were selected and tested. Totally 330 individual vines were examined for presence of viruses; dormant canes were sampled from these vines during winter (Burger and Thatcher 1987, Rowhani et al. 1992).

Viruses were detected using ELISA. Commercial antisera (Agritest, Italy) against GFLV, ArMV, GVA, GVB, GFkV, GLRaV-1 and GLRaV-3 were used in DAS-ELISA method according to instructions of manufacturer.

Microplates were coated with IgG to individual viruses diluted 1:1000 in coating buffer (1.59 g of Na_2CO_3, 2.93 g of $NaHCO_3$ and 0.2 g of NaN_3, dilute to 1 l with distilled water, adjust pH = 9.6). Reaction volume was 200 µl. Plates were incubated 4 hours at 37°C, washed 3 times with PBS (8 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4, 2.9 g of $Na_2HPO_4.12\ H_2O$, 0.2 g of NaN_3, 0.5 ml of Tween 20, add water to 1 l, adjust pH = 7.4) and samples (antigens) were added. Samples were prepared by grinding 0.55 g of phloem scrappings in 8.25 ml (ratio 1:15, w:v) of extraction buffer (PBS with 2% of PVP K-40 and 0.2% of BSA, adjust pH = 7.4). Commercially purchased negative and positive controls (Agritest, Italy) to individual viruses were used. All samples were performed in two wells. Plates were incubated overnight at 4°C, than washed 3 times and added alkaline phosphatase conjugated antibodies to individual viruses diluted 1:1000 in extraction buffer. Plates were incubated 4 hours at 37°C. In case of GVB and GFkV, monoclonal antibodies were used in this step, diluted 1:1000 in extraction buffer, after washing alkaline phosphatase conjugated antimouse antibodies diluted 1:1000 in extraction buffer were added and incubated 4 hours at 37°C. All plates were then washed 3 times with PBS and

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Table 1. Occurrence of viruses in Czech propagation material of grapevine varieties and rootstocks

Variety	GFLV	ArMV	GVA	GVB	GFkV	GLRaV-1	GLRaV-3
Agni	–	+	+	+	+	+	–
Alibernet	–	–	–	–	+	–	–
Andre	–	+	–	+	–	–	–
Ariana	–	–	–	–	–	–	–
Arkadia	–	–	–	–	–	–	–
Aurelius	–	+	–	–	–	–	+
Cabernet Moravia	–	+	–	–	–	–	–
Cabernet Sauvignon	+	+	+	–	+	–	+
Gewürztraminer	–	+	–	–	–	+	–
Chardonnay	–	+	–	+	–	–	–
Chasselas Blanc	–	–	–	–	–	–	–
Chasselas Rouge	–	–	–	–	–	–	+
Irsay Oliver	–	–	–	–	–	–	–
Julski Biser	–	+	–	–	+	–	–
Laurot	–	–	–	–	–	+	–
Limberger	+	+	+	–	+	+	–
Malvasier	+	+	+	+	+	+	–
Malverina	–	+	–	–	–	+	–
Muscat of Moravia	+	–	–	–	–	+	+
Muscat Ottonel	+	–	+	–	–	+	–
Müller-Thurgau	+	+	–	+	–	+	+
Neronet	–	–	–	–	–	–	–
Neuburger	+	+	–	–	–	–	–
Olšava	+	–	–	–	–	–	–
Palava	+	+	–	+	–	–	–
Panonia Kincse	–	–	–	–	–	–	–
Pinot Blanc	+	+	–	–	+	+	–
Pinot Gris	+	+	–	–	–	–	+
Pinot Noir	+	–	+	–	–	–	–
Pola	+	–	–	–	–	–	–
Portugais Bleu	+	+	–	+	+	+	–
Riesling	–	+	–	–	+	–	–
Saint Laurent	+	–	–	+	+	+	+
Sauvignon Blanc	+	+	+	+	+	+	+
Silvaner	+	+	+	–	+	+	+
Veltliner	–	–	–	–	–	–	+
Veritas	–	–	–	–	–	–	–
Vitra	–	+	–	–	–	–	–
Welschriesling	+	+	–	–	+	+	+
Zweigeltrebe	–	+	+	–	+	+	+
Amos	+	+	+	+	–	+	–
Craciunel 2	–	+	+	–	–	–	+
K 1	–	–	–	–	–	–	+
Kober 125 AA	+	+	+	–	–	–	+
Kober 5 BB	–	–	–	–	–	–	–
SO 4	+	+	–	+	–	–	–
Teleki 5C	+	+	–	+	–	+	–
Infected clones totally	22	44	15	13	18	29	20
Infected vines totally	23	63	16	13	25	34	25

GFLV – *Grapevine fanleaf virus*, ArMV – *Arabis mosaic virus*, GVA – *Grapevine virus A*, GVB – *Grapevine virus B*, GFkV – Grapevine fleck virus, GLRaV-1 – Grapevine leafroll-associated virus 1, GLRaV-3 – *Grapevine leafroll-associated virus 3*
+ positive, virus detected, – negative, virus not detected

added substrate buffer (97 ml of diethanolamine, 0.2 g of NaN_3, adjust pH to 9.6 with HCl, dilute with distilled water to 1 l) with 10 mg/ml of p-nitrophenylphosphate. In case of positive samples yellow colour of substrate developed. Absorbances at 405 nm were measured after two hours and were considered positive when the mean absorbance was at least three standard deviation units above the negative control.

RESULTS

Presence of individual viruses is summarized in Table 1.

Presence of GFLV was confirmed in varieties Cabernet Sauvignon, Limberger, Malvasier, Muscat of Moravia, Muscat Ottonel, Müller-Thurgau, Neuburger, Olšava, Palava, Pinot Blanc, Pinot Gris, Pinot Noir, Pola, Portugais Bleu, Saint Laurent, Sauvignon Blanc, Silvaner, Welschriesling and in rootstocks Amos, Kober 125 AA, SO 4 and Teleki 5C. Totally 23 vines of 22 clones were infected with this virus.

ArMV was found in varieties Agni, Andre, Aurelius, Cabernet Moravia, Cabernet Sauvignon, Gewürztraminer, Chardonnay, Julski Biser, Limberger, Malvasier, Malverina, Müller-Thurgau, Neuburger, Palava, Pinot Blanc, Pinot Gris, Portugais Bleu, Riesling, Sauvignon Blanc, Silvaner, Vitra, Welschriesling, Zweigeltrebe and in rootstocks Amos, Craciunel 2, Kober 125 AA, SO 4 and Teleki 5C. Totally 63 vines of 44 clones were infected with this virus.

GVA was detected in varieties Agni, Cabernet Sauvignon, Limberger, Malvasier, Muscat Ottonel, Pinot Noir, Sauvignon Blanc, Silvaner, Zweigeltrebe and in rootstocks Amos, Craciunel 2 and Kober 125 AA. Totally 16 vines of 15 clones were infected with this virus.

GVB occurred in varieties Agni, Andre, Chardonnay, Malvasier, Müller-Thurgau, Palava, Portugais Bleu, Saint Laurent, Sauvignon Blanc and in rootstocks Amos, SO 4 and Teleki 5C. Totally 13 vines of 13 clones were infected with this virus.

GFkV was found in varieties Agni, Alibernet, Cabernet Sauvignon, Julski Biser, Limberger, Malvasier, Pinot Blanc, Portugais Bleu, Riesling, Saint Laurent, Sauvignon Blanc, Silvaner, Welschriesling and Zweigeltrebe. Totally 25 vines of 18 clones were infected with this virus.

GLRaV-1 was found in varieties Agni, Gewürztraminer, Laurot, Limberger, Malvasier, Malverina, Muscat of Moravia, Muscat Ottonel, Müller-Thurgau, Pinot Blanc, Portugais Bleu, Saint Laurent, Sauvignon Blanc, Silvaner, Welschriesling, Zweigeltrebe and in rootstocks Amos and Teleki 5C. Totally 34 vines of 29 clones were infected with this virus.

GLRaV-3 was found in varieties Aurelius, Cabernet Sauvignon, Chasselas Rouge, Muscat of Moravia, Müller-Thurgau, Pinot Gris, Saint Laurent, Sauvignon Blanc, Silvaner, Veltliner, Welschriesling, Zweigeltrebe and in rootstocks Craciunel 2, K 1 and Kober 125 AA (Table 1). Totally 25 vines of 20 clones were infected with this virus.

No viruses were found in varieties Ariana, Arkadia, Chasselas Blanc, Irsay Oliver, Neronet, Panonia Kincse, Veritas and in rootstock Kober 5 BB.

From 109 clones tested, in 11 clones negative vines were not found-two clones of Cabernet Sauvignon, one clone of Chasselas Rouge, one clone of Julski Biser, four clones of Malvasier, one clone of Saint Laurent, one clone of Veltliner and one clone of rootstock K 1. In the rest 98 clones, at least one negative vine was found. Such vines were promoted as candidate plants into screenhouse for grapevine certification located in Faculty of Horticulture Lednice and they will be further tested by other methods (woody indicators, herbaceous indicators, polymerase chain reaction).

DISCUSSION

GFLV, ArMV, GVA, GVB, GFkV, GLRaV-1 and GLRaV-3 were found to be widely spread in Czech propagation material of grapevine and are considered as economically important grapevine viruses in the Czech Republic. ArMV and GLRaV-1 were found most frequently in grapevines in Czech Republic, occurred in more then 10% of examined vines. Small number of vines was found to be infected with GVA and GVB.

From 330 individual vines tested, 148 vines (45%) were found to be infected with at least one virus. Similarly Flak and Gangl (1994) found in Austria more than 30% of tested vines positive to at least one of five viruses tested, when most spread were GLRaV-1 and GLRaV-3.

In Hungary, Lehoczky et al. (1992) found 15 viruses in grapevines. In our conditions, we can expect similar situation and testing for further viruses is needed.

Harmfulness of grapevine viruses and their effect on growth and fertility of grapevine in our conditions is still to be determined. Probably, their impact is not as severe as in countries with warmer climate, where the infection with ArMV or GVB is mostly lethal within two years (Boscia and Demarinis 1998). However, every virus infection has some effect on growth and quality, and especially in long-term cultures as grapevine, it has great economical impact. Sanitation of infected Czech grapevine clones is needed in near future.

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ABSTRAKT

Hodnocení zdravotního stavu révy vinné v ČR

Byl proveden průzkum zdravotního stavu révy vinné v ČR se zaměřením na výskyt hospodářsky významných virů. Množitelský materiál 109 klonů révy vinné byl testován na přítomnost sedmi virů: virus roncetu révy vinné, virus mozaiky huseníku, virus révy vinné A, virus révy vinné B, virus skvrnitosti révy vinné, virus svinutky révy vinné 1 a virus svinutky révy vinné 3. Z dormantního réví bylo odebráno lýko a testováno DAS-ELISA. Z hodnocených sedmi virů se všechny hojně vyskytují v českém množitelském materiálu révy vinné. Z 330 jednotlivých keřů bylo 148 pozitivních alespoň na jeden virus. Z testovaných 109 klonů byl u 98 klonů nalezen alespoň jeden negativní keř. Tyto rostliny byly umístěny v technickém izolátu na Zahradnické fakultě Mendelovy zemědělské a lesnické univerzity v Lednici jako kandidátní rostliny a budou testovány dalšími metodami. U infikovaných klonů bude nutno zahájit proces ozdravování.

Klíčová slova: virus roncetu révy vinné; virus mozaiky huseníku; virus révy vinné A; virus révy vinné B; virus skvrnitosti révy vinné; virus svinutky révy vinné 1; virus svinutky révy vinné 3; certifikace; ELISA; množitelský materiál

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