Short Communication

DETECTION OF *GRAPEVINE PINOT GRIS VIRUS* IN CERTIFIED GRAPEVINE STOCKS IN MORAVIA, CZECH REPUBLIC

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SUMMARY

Twenty one grapevine mother plants used by nurseries for propagation in South Moravia, Czech Republic, were tested for the presence of *Grapevine Pinot gris virus* (GPGV) and other viruses by simplex and multiplex RT-PCR. GPGV was found in all vines tested and *Grapevine virus A*, *Grapevine fanleaf virus* and *Grapevine fleck virus* were detected only in some of them. Part of the movement and coat protein coding regions of 21 GPGV isolates was sequenced. Phylogenetic analysis revealed that south Moravian GPGV isolates grouped with isolates from other regions and countries. This study provides the first comprehensive survey of the GPGV occurrence in South Moravia.

Keywords: *Grapevine Pinot gris virus*, sequencing, RT-PCR

Grapevine Pinot gris virus (GPGV), a new member of the genus *Trichovirus*, was first discovered on *Vitis vinifera* L. in the Trentino region of Italy (Giampetruzzi *et al*., 2012). Symptoms of stunting, chlorotic mottling and leaf deformation were associated with GPGV on cvs Pinot gris and Traminer. Subsequently, the virus was described on cvs Chardonnay, Pinot noir, Glera and the table grape cultivars Black magic and Supernova (Saldarelli *et al*., 2014) in Italy, on cvs Merlot and Carignan in France (Beuve *et al*., 2015) and on cvs Sauvignonasse in Slovenia (Plesko *et al*., 2014). In the Slovak Republic, three complete and several partial GPGV genomes were sequenced (Glasa *et al*., 2014). GPGV infection was found on *V. vinifera* x *V. labrusca* cv. Tamnara in Siheung, Korea (Cho *et al*., 2013). These studies, besides showing a widespread occurrence of the virus in several European countries, highlighted its uncertain association with symptoms (Glasa *et al*., 2014) due to GPGV presence in asymptomatic plants. The possibility that the virus exists as a population of genetically distinct virulent and latent isolates was suggested in two independent studies (Saldarelli *et al*., 2015; Bianchi *et al*., 2015).

The aim of the present study was to broaden the knowledge about GPGV in the South Moravian region of the Czech Republic. The research focused on certified grapevine mother plants that can act as primary sources of the virus. The GPGV isolates used in this study were collected in 2013-2014 (Table 1).

RT-PCR detection of GPGV was done as described (Morelli *et al*., 2014). Simplex (Eichmeier *et al*., 2010; Minafra *et al*., 1992; Osman and Rowhani, 2006; Sabanadzovic *et al*., 1996; Sefc *et al*., 1999; Wetzel *et al*., 2002) and multiplex (Qualiplante, Clapiers, France) RT-PCR were performed to identify *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), *Grapevine leafroll-associated virus 3* (GLRaV-3), *Grapevine virus A* (GVA) and *Grapevine fleck virus* (GFkV) (Table 1). Simplex PCR reaction mix consisted of 10.5 µl of water (HPLC purity), 4 µl of 5× Colorless GoTaq® Flexi Buffer for polymerase (Promega, Madison, USA), 1.2 µl of 25 mM $MgCl_2$ (Promega, Madison, USA), 0.2 µl of 10 µM dNTP mixture (Invitek, Berlin, Germany), 0.2 µl of GoTaq® G2 Flexi DNA polymerase (5u/µl) (Promega, Madison, USA), 1 µl of each of primer pair (10 µM), 0.7 µl of Flexi 5× Green GoTaq® Flexi Buffer (Promega, Madison, USA) and 2 µl of cDNA template. PCR cycling consisted of an initial denaturation for 3 min at 95°C, 40 cycles at 95°C for 2 sec, 40 sec of primer annealing at 63°C (this temperature was dependent on the primer pair) and 2 min at 72°C for extension, followed by a final step at 72°C for 5 min. Multiplex RT-PCR was performed according to the protocol of Qualiplante.

GPGV genomic RNA regions were amplified from the 21 isolates using the primers reported by Morelli *et al*. (2014). Failure to sequence the 588 bp amplicons from all the 21 isolates prompted a manual alignment of sequences at a length of 288 bp.

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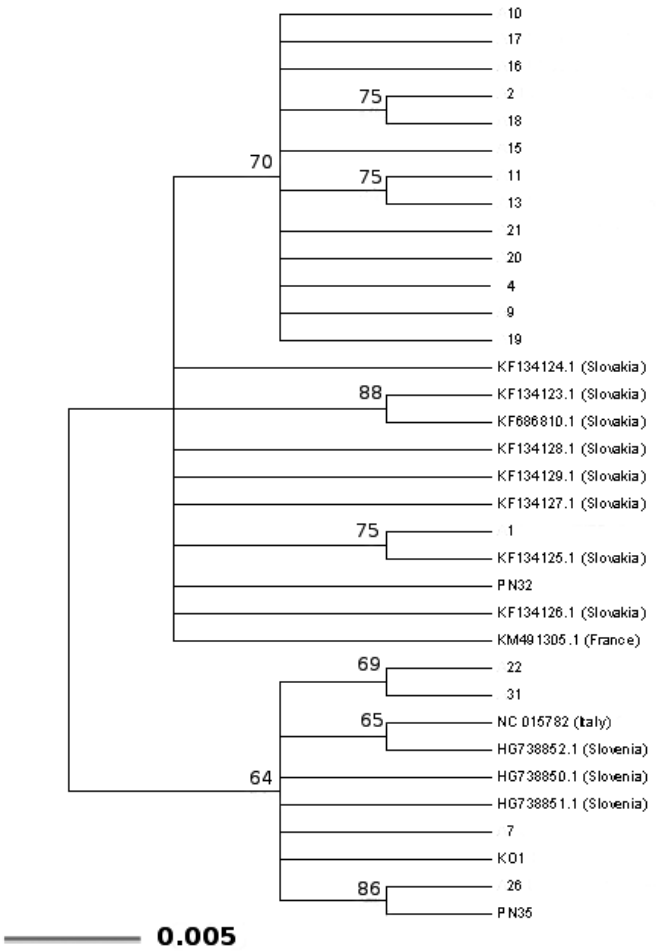

Fig. 1. Phylogenetic tree of Moravian GPGV isolates and other isolates for which sequence information is available in GenBank. Maximum likelihood method with 1000 bootstrap replicates was implemented in MEGA 5.2.2 (Tamura *et al*., 2011). All nodes supporting a threshold of 60%, are indicated. Scale bar represents units in nucleotide substitutions per site.

Nucleotide sequences were analysed by CLC Main Workbench 6.0 (CLC bio, Denmark), and deposited in GenBank as accession numbers KP715515-KP715524 and KP715526-KP715536.

The GPGV sequences showed identities of 91.86 to 100% at the nucleotide level. Phylogenetic analysis was performed on the 288 bp sequence of 21 South Moravian isolates and sequences from Italian (Giampetruzzi *et al*., 2012), Slovenian (Plesko *et al*., 2014), Slovakian (Glasa *et al*., 2014), and French (Beuve *et al*., 2015) isolates (Fig. 1). The tree topology showed two distinct clades of genetic variants. The majority of South Moravian isolates (67%, 14 of 21) clustered in clade 1 with isolates from Slovakia and France, and a few South Moravian isolates (33%, 7 of 21) clustered in clade 2 with isolates from Slovenia and Italy (Fig. 1).

This study confirms the widespread occurrence of GPGV in the most significant wine-producing region in the Czech Republic and that suggests GPGV dissemination can be due to the use of infected propagation material.

Table 1. RT-PCR detection of GPGV and three other viruses in South Moravian certified grapevine mother plants. GenBank accession numbers of GPGV isolates, and positive (+) and negative (–) RT-PCR results are indicated.

Sample	GenBank Acc. Nos.	Cultivar	GPGV	GVA	GFkV	GFkV
KO1	KP715515	Kodrjanka	+	–	–	+
PN32	KP715516	Pamjati Negrula	+	–	–	+
PN35	KP715517	Pamjati Negrula	+	+	–	+
1	KP715518	Müller Thurgau	+	–	+	–
2	KP715519	Müller Thurgau	+	+	+	–
4	KP715520	Müller Thurgau	+	+	+	–
7	KP715521	Chardonnay	+	+	+	–
9	KP715522	Chardonnay	+	–	+	–
10	KP715523	Chardonnay	+	–	+	–
11	KP715524	Chardonnay	+	–	+	–
13	KP715526	Chardonnay	+	–	+	–
15	KP715527	Chardonnay	+	–	+	–
16	KP715528	Chardonnay	+	–	+	–
17	KP715529	Chardonnay	+	–	+	–
18	KP715530	Chardonnay	+	–	+	–
19	KP715531	Chardonnay	+	–	+	–
20	KP715532	Chardonnay	+	–	+	–
21	KP715533	Chardonnay	+	+	+	–
22	KP715534	Chardonnay	+	+	+	–
26	KP715535	Chardonnay	+	+	+	–
31	KP715536	Chardonnay	+	–	+	–

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