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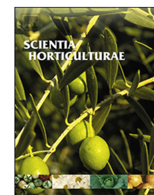

Short Communication

Detection and distribution of *Grapevine rupestris stem pitting-associated virus* in grapevine

Guojun Hu, Yafeng Dong*, Hongjuan Zhu, Zunping Zhang, Xudong Fan, Fang Ren

National Center for Eliminating Viruses from Deciduous Fruit Trees, Research Institute of Pomology, Chinese Academy of Agricultural Sciences, Xingcheng 125100, PR China

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A B S T R A C T

Grapevine rupestris stem pitting-associated virus (GRSPaV) is a widespread virus of grapevine. In this study, we compared the detection efficiencies of two primer sets and evaluated virus distribution among different grapevine materials. The results of RT-PCR analyses showed that primer pair RSP52/53 was 10% more sensitive than RSP9F9R, with total detection rates of 89.3% and 78.6%, respectively. Five different parts of six grapevine cultivars, which were collected in May and August, were used as materials in GRSPaV detection analyses. The results showed that the fourth petioles were a suitable material for analysis, and fourth petiole materials collected in May and August yielded the same result (50.0%). The plant parts could be ranked, from highest virus detection efficiency to lowest, as follows: phloem > berries ≈ fourth petioles > tendrils > new leaves ≈ fourth leaves. A comparison of the titers of GRSPaV among the upper, middle, and lower above-ground parts and roots of three *in vitro* grapevine samples by quantitative real-time PCR showed that the highest concentration of GRSPaV was in the root, followed by the upper above-ground part.

1. Introduction

Grapevine is one of the most widely grown fruit crops worldwide. Its prolonged history of cultivation and the more recent practice of grafting in viticulture have led to a great number of viruses infecting grapevine (Meng et al., 2006). More than 70 viruses and virus-like species can infect grapevine, and fan-leaf degeneration, leaf-roll, fleck, and rugose wood disorders are the most important grapevine diseases (Martelli, 2014). Among them, rugose wood disorder is one of the most widespread graft-transmissible diseases of grapevine. This disease is associated with four vitiviruses (*Grapevine virus A*, *Grapevine virus B*, *Grapevine virus D,* and *Grapevine virus E*) and the foveavirus *Grapevine rupestris stem pitting-associated virus* (GRSPaV) (Beuve et al., 2013). GRSPaV is perhaps the most prevalent virus of grapevines. It has been detected in many different grapevine genotypes and occurs worldwide (Meng and Gonsalves, 2003). In recent years, GRSPaV has been found for the first time in Russia (Dmitrenko et al., 2016), Korea (Jo et al., 2017), and Tunisia (Selmi et al., 2017). GRSPaV is associated with several diseases, namely rupestris stem pitting, vein necrosis, and Syrah decline, and it may also be involved in other disorders (Meng et al., 1998; Bouyahia et al., 2005; Morelli et al., 2011; Lima et al., 2006; 2009). GRSPaV is spread by vegetative propagation, grafting, and possibly *via* seeds, and is restricted to grapevines. No biological vectors have been reported (Meng and Gonsalves, 2003).

GRSPaV is a positive sense single-stranded RNA virus with an 8.7-kb genome that is polyadenylated at the 3′ terminus (Martelli and Jelkmann, 1998). The virus contains five open reading frames (ORFs) potentially encoding proteins involved in replication (ORF1), the triple gene block (TGBp) 1, 2, and 3 (ORF 2, 3, and 4), and the coat protein (ORF5) (Meng and Gonsalves, 2003). Depending on their full genomic sequences, the variants of GRSPaV can be divided into three main clusters: cluster I (GRSPaV-SY), cluster II comprising sub-cluster IIa (GRSPaV-SG1, GRSPaV-MG) and sub-cluster IIb (GRSPaV-1), and cluster III (GRSPaV-BS, GRSPaV-PN) (Beuve et al., 2013). Recently, a new cluster (IV, GRSPaV-JF) was reported (Hu et al., 2015). GRSPaV exhibits extensive genetic diversity and has numerous sequence variants. Different strains of GRSPaV are found in different grapevine varieties and even in different materials from the same plant. It has been reported that one grapevine plant can harbor at least four major groups of GRSPaV variants, with two to four distinct viral variants in the scion and one variant in the rootstock (Meng et al., 2006). It is possible that different GRSPaV strains are responsible for different

Abbreviations: GRSPaV, *Grapevine rupestris stem pitting-associated virus*; ORFs, open reading frames; qPCR, Quantitative real-time PCR; TGBp, the triple gene block protien; UTR, untranslated region

* Corresponding author.
E-mail address: yfdong@163.com (Y. Dong).

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diseases (Meng et al., 2013).

China is one of the main grapevine-producing countries, and previous studies have shown that the occurrence of GRSPaV is widespread in Chinese grapevines (Fan et al., 2012; Zhu et al., 2014; Hu et al., 2015). In our research, we have found that the efficiency of virus detection is affected by the sensitivity of the primers and type of materials analyzed. In this study, two primer pairs were chosen to detect GRSPaV and the detection efficiencies and virus distribution among different grapevine materials were compared.

2. Materials and methods

2.1. Plant materials

Shoots of 84 samples from eight provinces and regions of China were used to analyze the sensitivity of primers to detect GRSPaV (Supplementary Table S1). New leaves, tendrils, the fourth petioles, the fourth leaves, and berries of six cultivars (Cabernet Gernischt, Centennial Seedless, Xiangyue, Red Globe, Xiangfei, and Cabernet Sauvignon) were collected from the Research Institute of Pomology, Chinese Academy of Agriculture Sciences, Liaoning Province, in May and August. Three *in vitro* grapevine cultivars (Kyoho, Jumeigui, 87-1) were also used for the distribution analysis of GRSPaV. These samples were divided into four parts, among them, the above-ground tissues were averagely divided three parts (upper, middle, and lower above-ground parts) and the below-ground tissues were the root part (Table 1). All the leaves and stems of the three above-ground parts were used for the virus detection. Each cultivar was examined in triplicate.

2.2. RNA extraction

Total RNA was extracted from different grapevine materials as described by Foissac et al. (2001) with some modifications. Plant samples (0.1 g) were homogenized in 1 mL grinding buffer. The clarified homogenate was added to 150 μL 10% *N*-lauroyl sarkosyl, the mixture was incubated at 72 °C for 10 min, and then on ice for 5 min. The mixture was centrifuged at 13,000 × *g* at 4 °C for 10 min, and 300 μL of the supernatant was added to the following mixture: 300 μL 6 M NaI, 150 μL ethanol, and 25 μL autoclaved silica powder suspension. After washing twice in half-strength washing buffer, RNA was eluted in 100 μL nuclease-free water, then incubated at 72 °C for 4 min. After centrifugation, nucleic acids were stored at −72 °C until further analysis.

2.3. RT-PCR

First-strand cDNA synthesis was conducted in a 20-μL reaction system. The mixture contained 1 μg RNA, 4 μL M-MLV RT 5× buffer, 0.5 mM dNTP mix (TaKaRa, Dalian, China), 5 μM random hexamer primers (Sangon Biotech, Shanghai, China), and 5 U M-MLV reverse transcriptase (Promega, Madison, WI, USA). The mixture was incubated at 37 °C for 10 min and then at 42 °C for 50–60 min. The PCR was carried out in a 25-μL reaction volume containing 2.5 μL 10× PCR buffer (Mg^{2+}), 0.5 μL 10 mM dNTPs, 0.5 μL each primer (final concentration 1 μM), 0.125 μL 5 U/μL r*Taq* DNA polymerase (TaKaRa), 2 μL template cDNA, and 18.8 μL sterile water. The primer pairs used to amplify the viral sequences are shown in Table 2.

The cycling profile was as follows: pre-denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30–40 s, and extension at 72 °C for 40–50 s, with final extension at 72 °C for 5–7 min. Amplification was conducted in a 96-well PCR Thermal Cycler (Bio-Rad S1000, Bio-Rad, Hercules, CA, USA). Amplicons were separated by electrophoresis on 1.2% (w/v) agarose gels, stained with ethidium bromide, and then visualized under ultraviolet light.

2.4. Quantitative real-time PCR (qPCR)

Total RNA samples were treated with a RT reagent kit with gDNA Eraser (TaKaRa) to remove genomic DNA. First-strand cDNA was synthesized according to the manufacturer's instructions and Hu et al. (2018).

The titer of GRSPaV in different materials was quantified using SYBR® Premix Ex Taq™ II (TaKaRa) according to the manufacturer's instructions for the CFX Connect real-time system (Bio-Rad). The 20-μL reaction mixtures were incubated at 95 °C for 2 min for initial denaturation, followed by 40 cycles at 95 °C for 10 s, 58 °C for 15 s, and 72 °C for 20 s. The ΔCt method was used to calculate the relative concentration of GRSPaV. Each sample was examined in triplicate. The primer pair Vivi-18S-f/r was used as an internal control (Table 2).

3. Results

3.1. Sensitivity of GRSPaV detection primers

The phloem of canes collected from grapevine was used for RNA extraction. The detection rate of GRSPaV in 84 grapevine samples was 98.8%, as detected by the two primer pairs RSP9F/9R and RSP52/53 (Table 2). The RT-PCR results showed that the sensitivity of the two primer pairs was different. GRSPaV was detected in 57 samples by both of the primer pairs. The total detection rate of primer pair RSP52/53 was 89.3% and that of RSP9F9R was 78.6%; therefore, RSP52/53 was 10% more sensitive than RSP9F9R in detecting GRSPaV (Table 3).

3.2. GRSPaV detection efficiency in different parts of grapevine collected in May and August

To analyze the efficiency of GRSPaV detection in different materials, we tested the new leaves, tendrils, fourth petioles, fourth leaves, and berries of six cultivars that had been confirmed to be GRSPaV-positive by phloem analyses. These materials were collected in May and August (berries were only acquired in August) and the primer pair RSP52/53 was used in the PCR analysis. The detection efficiencies differed among the five parts of grapevine and all of them were lower than that of the phloem (Fig. 1). GRSPaV could hardly be detected in new leaves and the fourth leaves in the two periods. GRSPaV was detected in the tendrils collected in August for one out of three cultivars, but in none of the tendrils collected in May. The detection efficiency in the fourth petioles was consistent between the two periods, with a rate of 50.0%. The detection rate in berries collected in August was the same as that in the fourth petioles. There were also cultivar-specific differences in detection. GRSPaV could be detected in the fourth petioles of Cabernet Gernischt but not those of Cabernet Sauvignon, and *vice versa* in berries.

3.3. Distribution of GRSPaV in different parts of in vitro grapevine

Three cultivars were used in these analyses. For each cultivar, five *in*

Table 1
Details of grapevines used in *Grapevine Rupestris stem pitting associated virus* detection analysis.

Cultivars	Species	Origins
Cabernet Gernischt	*Vitis vinifera* L.	Ningxia, China
Centennial Seedless	*V. vinifera* L.	Xinjiang, China
Xiangyue	*V. vinifera* L.	Liaoning, China
Red Globe	*V. vinifera* L.	Liaoning, China
Xiangfei	*V. vinifera* L.	Liaoning, China
Cabernet Sauvignon	*V. vinifera* L.	Shandong, China
Kyoho	*V. vinifera* L.	Liaoning, China
87-1	*V. vinifera* L.	Liaoning, China
Jumeigui	*V. vinifera* L.	Liaoning, China

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