

## Incidence of 14 grapevine viruses in Korean vineyards

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### Abstract

The incidence of grapevine virus infections in Korean vineyards was investigated from July to October, 2020. A total of 177 petiole samples were collected from two or three different cultivars in each of four different regions; these were examined by reverse transcription-polymerase chain reaction assay for the presence of 14 major viruses. The overall occurrence of grapevine viruses was 91.0%, and the level of incidence was high irrespective of region or cultivar. The predominant viruses were *grapevine leafroll-associated virus 3* (80.2%), *grapevine fleck virus* (70.6%), and *grapevine rupestris stem pitting-associated virus* (49.2%). Most grapevines were infected with multiple viruses, suggesting that Korean vineyards are likely to suffer economic losses resulting from viral diseases. This is the first extensive survey performed in Korea to observe the outbreak status of diverse grapevine viruses; surveys of this type can provide important information for the management of grapevine viruses in Korea.

**Keywords:** clean stock; rugose wood complex; RT-PCR; *Vitis*; virus outbreak

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### Introduction

Because they inhibit photosynthesis and anthocyanin biosynthesis, viral diseases are a major factor negatively affecting fruit yield and quality in grapevines (Martínez-Lüscher *et al.*, 2019; Tobar *et al.*, 2020; Lee *et al.*, 2021). In addition, virus infection can decrease freezing tolerance, leading to increased susceptibility to winter vine damage, or even to death (Xiao *et al.*, 2018). The impacts of viral diseases suggest the importance of establishing a system of virus-free grapevine cultivation to maintain the competitiveness of the grapevine industry.

Grapes are cultivated in Korea for the fresh fruit market, and for the production of wine and other drinks (Heo *et al.*, 2016; Lee *et al.*, 2019). They are the fifth most important fruit crop in terms of growing area, with a total area of 12,676 ha in 2019. Despite the crop's commercial importance, limited research regarding viral diseases of grapevines has been performed in Korea. Globally, 86 different viruses have to date been identified in grapevines (Fuchs, 2020). Some of the viruses causing significant economic loss are the *grapevine fleck virus* (GFkV), *grapevine asteroid mosaic-associated virus* (GAMaV), *grapevine rupestris vein feathering virus* (GRVFV), *grapevine fanleaf virus* (GFLV), *grapevine leafroll-associated viruses* (GLRaV-1, -2, -3, -4, -7, -13), *grapevine virus A* (GVA), *grapevine virus B* (GVB) and *grapevine rupestris stem pitting-associated virus* (GRSPaV). Although some of these viruses have been found in Korean vineyards (Jo *et al.*, 2018; Cho *et al.*,

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2018), detailed information on their occurrence and distribution is not available. For this reason, we investigated the presence and distribution of the 14 most important grapevine viruses in major grape cultivars in Korea.

## Materials and Methods

### *Biological material*

A total of 177 samples were collected from four major grape-growing regions in Korea from July to October 2020. In Korea, the most widely planted grape cultivars are: ‘Campbell Early’, ‘Kyoho’, and ‘Shine Muscat’, accounting for 38.6%, 21.4%, and 28.9% of the total grape cultivation area, respectively. These cultivars account for approximately 90% of the Korean grapevine market. The cultivation area of ‘Shine Muscat’, which was recently introduced to Korea, has sharply increased; it is expected to be the number one cultivar in the near future. Accordingly, most of the samples in the present study were collected from the ‘Shine Muscat’ cultivar.

### *Experimental procedures*

For virus detection, two leaf petioles from each of three vines per vineyard were collected and processed within one day of collection. A reverse transcription (RT)-PCR assay was used to evaluate the presence of each of 14 viruses, with each sample being tested individually. Total RNA was extracted from 50 mg of petioles using the TaKaRa MiniBEST Plant RNA Extraction Kit (Takara Bio, Tokyo, Japan) according to the manufacturer’s instructions. Final elution was performed using 30  $\mu$ L of RNase-free H<sub>2</sub>O. RNA purity and yield were assessed using a Nabi NanoDrop spectrophotometer (MicroDigital, Seongnam, Korea). The first strand of cDNA was synthesized using the PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio, Tokyo, Japan). PCR amplification were performed using AccuPower HotStart PCR PreMix (Bioneer, Daejeon, Korea) with two  $\mu$ L of cDNA and 10 pmol of each virus-specific primer, as designed by Xiao *et al.* (2018), in a 20  $\mu$ L final volume. Amplifications were carried out in a TP 350 thermal cycler (Takara Bio, Tokyo, Japan) under the following protocol: initial denaturation at 95 °C for 1 min, followed by 33 cycles of 30 s denaturation at 95 °C, 30 s at the annealing temperature specified for each primer, 1 min elongation at 72 °C, with a final extension at 72 °C for 5 min. PCR amplicons were checked by gel electrophoresis on 1.5% agarose gels and visualized with a GD-1000 gel documentation system (Axygen, California, USA).

## Results and Discussion

Viruses detected by RT-PCR, and details of their occurrence, are presented in Tables 1, 2, and 3. From 177 samples, the viruses detected were GLRaV-3 (80.2%), GFkV (70.6%), GRSPaV (49.2%), GVA (18.1%), GVB (13.6%), GPGV (7.3%), GLRaV-1 (6.8%), GLRaV-2 (6.2%), GRVFV (4.5%), GSyV-1 (4.5%), and GAMaV (4.0%). The overall virus incidences in Cheonan, Gimcheon, Hwaseong, and Yeongwol were 87.5%, 91.1%, 96.3%, and 86.7%, respectively, and there were no significant differences among the regions. Similarly, overall rates of virus infection were very high, regardless of cultivar: 84.1% in ‘Campbell Early’, 77.8% in ‘Kyoho’, and 100% in ‘Shine Muscat’. Differences were observed, however, in single and mixed viral infection rates among different cultivars.

**Table 1.** Incidence of grapevine viruses in 4 regions

Region	No. of tested vines	No. of vines infected with virus	Percentage of vines infected with virus (%)
Cheonan	48	42	87.5
Gimcheon	45	41	91.1
Hwaseong	54	52	96.3
Yeongwol	30	26	86.7
Total	177	161	91.0

**Table 2.** Number and infection rates of analyzed vines in three different cultivars

Cultivar	Region	No. of tested vines	Viruses / Percentage of vines infected with each virus (%)													
			GAMa V	GFkV	GFLV	GLRa V-1	GLRa V-2	GLRa V-3	GLRa V-4	GLRa V-7	GPGV	GRSP aV	GRVF V	GSV-1	GVA	GVB
'Campbell Early'	Cheonan	18	0 (0.0)	3 (16.7)	0 (0.0)	2 (11.1)	0 (0.0)	13 (72.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)
	Hwaseong	24	0 (0.0)	8 (33.3)	0 (0.0)	3 (12.5)	1 (4.2)	20 (83.3)	0 (0.0)	0 (0.0)	0 (0.0)	7 (29.2)	1 (4.2)	1 (4.2)	0 (0.0)	0 (0.0)
	Yeongwol	21	0 (0.0)	9 (42.9)	0 (0.0)	0 (0.0)	0 (0.0)	14 (66.7)	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)	0 (0.0)	2 (9.5)	0 (0.0)	1 (4.8)
	<b>Subtotal</b>	<b>63</b>	<b>0 (0.0)</b>	<b>20 (31.8)</b>	<b>0 (0.0)</b>	<b>5 (7.9)</b>	<b>1 (1.6)</b>	<b>47 (74.6)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>1 (1.6)</b>	<b>7 (11.1)</b>	<b>2 (3.2)</b>	<b>3 (4.8)</b>	<b>0 (0.0)</b>	<b>1 (1.6)</b>
'Kyoho'	Cheonan	9	3 (33.3)	7 (77.8)	0 (0.0)	1 (11.1)	1 (11.1)	5 (55.6)	0 (0.0)	0 (0.0)	0 (0.0)	5 (55.6)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)
	Gimcheon	18	0 (0.0)	11 (61.1)	0 (0.0)	1 (5.6)	2 (11.1)	9 (50.0)	0 (0.0)	0 (0.0)	1 (5.6)	2 (11.1)	0 (0.0)	3 (16.7)	0 (0.0)	0 (0.0)
	<b>Subtotal</b>	<b>27</b>	<b>3 (11.1)</b>	<b>18 (66.7)</b>	<b>0 (0.0)</b>	<b>2 (7.4)</b>	<b>3 (11.1)</b>	<b>14 (51.9)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>1 (3.7)</b>	<b>7 (25.9)</b>	<b>0 (0.0)</b>	<b>5 (18.5)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>
'Shine Muscat'	Cheonan	21	0 (0.0)	21 (100)	0 (0.0)	1 (4.8)	4 (19.1)	19 (90.5)	0 (0.0)	0 (0.0)	2 (9.5)	21 (100)	2 (9.5)	0 (0.0)	10 (47.6)	5 (23.8)
	Hwaseong	30	3 (10.0)	30 (100)	0 (0.0)	2 (6.7)	1 (3.3)	29 (96.7)	0 (0.0)	0 (0.0)	4 (13.3)	21 (70.0)	1 (3.3)	0 (0.0)	8 (26.7)	6 (20.0)
	Gimcheon	27	1 (3.7)	27 (100)	0 (0.0)	2 (7.4)	2 (7.4)	24 (88.9)	0 (0.0)	0 (0.0)	5 (18.6)	24 (88.9)	2 (7.4)	0 (0.0)	10 (37.0)	10 (37.0)
	Yeongwol	9	0 (0.0)	9 (100)	0 (0.0)	0 (0.0)	0 (0.0)	9 (100)	0 (0.0)	0 (0.0)	0 (0.0)	7 (77.8)	1 (11.1)	0 (0.0)	4 (44.4)	2 (22.2)
	<b>Subtotal</b>	<b>87</b>	<b>4 (4.6)</b>	<b>87 (100)</b>	<b>0 (0.0)</b>	<b>5 (5.8)</b>	<b>7 (8.1)</b>	<b>81 (93.1)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>11 (12.6)</b>	<b>73 (83.9)</b>	<b>6 (6.9)</b>	<b>0 (0.0)</b>	<b>32 (36.8)</b>	<b>23 (26.4)</b>
<b>Total</b>	<b>177</b>	<b>7 (4.0)</b>	<b>125 (70.6)</b>	<b>0 (0.0)</b>	<b>12 (6.8)</b>	<b>11 (6.2)</b>	<b>142 (80.2)</b>	<b>0 (0.0)</b>	<b>0 (0.0)</b>	<b>13 (7.3)</b>	<b>87 (49.2)</b>	<b>8 (4.5)</b>	<b>8 (4.5)</b>	<b>32 (18.1)</b>	<b>24 (13.6)</b>	

**Table 3.** Cultivar infection rates with multiple-virus combinations

Cultivar	Region	No. of tested vines	No. of viruses in mixed infections / number of vines with multiple viruses (%)						
			0	1	2	3	4	5	6
'Campbell Early'	Cheonan	18	4(22.2)	9(50.0)	5(27.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Hwaseong	24	2(8.3)	8(33.3)	9(37.5)	5(20.8)	0(0.0)	0(0.0)	0(0.0)
	Yeongwol	21	4(19.1)	9(42.9)	6(28.6)	2(9.5)	0(0.0)	0(0.0)	0(0.0)
	<b>Total</b>	<b>63</b>	<b>10(15.9)</b>	<b>26(41.3)</b>	<b>20(31.8)</b>	<b>7(11.1)</b>	<b>0(0.0)</b>	<b>0(0.0)</b>	<b>0(0.0)</b>
'Kyoho'	Cheonan	9	2(22.2)	0(0.0)	2(22.2)	1(11.1)	3(33.3)	1(11.1)	0(0.0)
	Gimcheon	18	4(22.2)	6(33.3)	3(16.7)	3(16.7)	2(11.1)	0(0.0)	0(0.0)
	<b>Total</b>	<b>27</b>	<b>6(22.2)</b>	<b>6(22.2)</b>	<b>5(18.5)</b>	<b>4(14.8)</b>	<b>5(18.5)</b>	<b>1(3.7)</b>	<b>0(0.0)</b>
'Shine Muscat'	Cheonan	21	0(0.0)	0(0.0)	1(4.8)	5(23.8)	8(38.1)	6(28.6)	1(4.8)
	Hwaseong	30	0(0.0)	0(0.0)	2(6.7)	17(56.7)	6(20.0)	4(13.3)	1(3.3)
	Gimcheon	27	0(0.0)	0(0.0)	2(7.4)	6(22.2)	12(44.4)	5(18.5)	2(7.4)
	Yeongwol	9	0(0.0)	0(0.0)	1(11.1)	4(44.5)	2(22.2)	2(22.2)	0(0.0)
	<b>Total</b>	<b>87</b>	<b>0(0.0)</b>	<b>0(0.0)</b>	<b>6(6.9)</b>	<b>32(36.8)</b>	<b>28(32.2)</b>	<b>17(19.5)</b>	<b>4(4.6)</b>

In the 'Shine Muscat' cultivar, all samples were infected with at least one virus, and 11 different viruses were detected across the regions. In the single infection pattern, GFkV, GLRaV-3, and GRSPaV infected 100.0%, 93.1%, and 83.9%, respectively, of the vines tested. Relatively high incidences of GVA (36.8%) and GVB (26.4%) were also observed. Although GFLV, GLRaV-4, GLRaV-7, and GSyV-1 were not detected in this study, infections of GAMaV, GLRaV-2, GPGV, and GRVfV were also observed, and multiple viral infections were common. There were 26 different combinations of mixed virus infections, with 6.9%, 36.8%, 32.2%, 19.5%, and 4.6% of the samples being infected with two, three, four, five, and six viruses, respectively. Triple infections with GLRaV-3 + GFkV + GRSPaV were recorded most often (28.7%), followed by quadruple infections with GLRaV-3 + GFkV + GRSPaV + GVA (10.3%), and quintuple infections with GLRaV-3 + GFkV + GRSPaV + GVA + GVB (10.3%).

In the 'Campbell Early' cultivar, nine viruses were detected, the most frequent being GLRaV-3 (74.6%), followed by GFkV (31.8%), then GRSPaV (11.1%). Interestingly, most samples tested were infected by one or two viruses, and only 11.1% of samples were triply infected. The most frequent virus combination was GLRaV-3 + GFkV (14.3%), followed by GLRaV-3 + GRSPaV (7.9%), then GLRaV-1 + GLRaV-3 (3.2%). Eight different viruses were detected in Kyoho. GFkV (66.7%) was the predominant virus, with GLRaV-3 and GRSPaV being also found frequently. A total of 18.5% of samples were doubly infected, 14.8% of samples were triply infected, 18.5% were quadruply infected, and 3.7% were quintuply infected. The dominant mixed virus infection was GLRaV-3 + GFkV (14.8%), followed by GLRaV-3 + GFkV + GRSPaV + GSyV-1 (7.4%). Other multiple infections were less common, with an incidence of less than 4%. The major viruses in 'Campbell Early', 'Kyoho', and 'Shine Muscat' were therefore similar, but fewer mixed infections were found in the 'Campbell Early' and 'Kyoho' cultivars compared to 'Shine Muscat'.

Our study indicates that the degree of virus occurrence in Korean vineyards is disturbing. Commonly occurring viruses across three different grape cultivars were GFkV, GLRaV-3, and GRSPaV. GLRaV-3 is a phloem-restricted virus (Hu *et al.*, 2020) that is predominant in vineyards worldwide, including Canada and the United States (Poojari *et al.*, 2020; Schoelz *et al.*, 2021). It hinders photosynthesis and carbon balance during respiration and induces a significant economic loss by reducing the vigor and yield of vines (Endeshaw *et al.*, 2014; Montero *et al.*, 2016). Mixed infection with other viruses also leads to commercially-significant problems in berry ripening and cluster size (Lee and Martin, 2009), and GLRaV-3 is regarded as one of the most detrimental viruses. GLRaV-3 is mainly disseminated by propagation using virus-infected vines, but subsequent spread is mediated by mealybugs or soft scale insects (Golino *et al.*, 2008). The frequent occurrence of GFkV and GRSPaV was remarkable and believed to be explained by the use of infected cuttings in vegetative propagation or by grafting with infected rootstocks because no natural vector is known (Crnogorac *et al.*, 2021). Although a single infection of this kind of virus does not significantly affect vine growth, it is suspected that mixed infections with other viruses could negatively affect vine vigor, fruit yield, and juice quality. Our results show that mixed infections among GFkV, GRSPaV, and other viruses frequently occur in Korean vineyards, and to avoid economic loss should be taken into consideration when evaluating potential hazards to productivity and grape quality in Korea.

Several viruses-such as GLRaV-3, GVA, and GVB-can be transmitted by vectors in Korean vineyards. A strong relationship between the incidence of specific viruses and the magnitude of vector populations is frequently reported (Cooper *et al.*, 2018; Jones and Nita, 2019; Uhls *et al.*, 2021), but there is no information on virus infection routes and management practices in Korean vineyards. These circumstances suggest that a lack of monitoring and prevention systems for viral vectors could contribute to these vineyard viruses. For this reason, it is important that efforts be made to obtain information on virus vectors in Korean vineyards.

We found differences in virus occurrence among cultivars, which might have resulted from different exposure conditions to virus infection, but equally could be due to different susceptibilities, among cultivars, to specific viruses. Along with the great success of the Shine Muscat cultivar, new grape cultivars are continuously being released in Korea (Heo *et al.*, 2017; Roh *et al.*, 2018; Park *et al.*, 2020) and the

diversification of grape-cultivation cultivars is expected to progress in the future. Based on our surveys, thorough virus screening should be performed before vine material is released for propagation.

## Conclusions

Our study has demonstrated the local grapevine industry in Korea is exposed to a serious risk caused by viruses. In this study, GLRaV-3, GFkV and GRSPaV that can influence growth and quality of grapevine negatively are commonly found regardless of cultivars and regions, which emphasize the need to develop strategies for eliminating such viruses and preventing their spread.

## Authors' Contributions

JYH conceived and designed the experiments, supervised, and revised the manuscript. SHK conducted the experiments and wrote the original manuscript. SHJ conducted parts of the experiments. All authors read and approved the final manuscript.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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