Short Communication

North American Grape ‘Norton’ is Resistant to Grapevine Vein Clearing Virus

Wenping Qiu,[†] Sylvia M. Petersen, and Susanne Howard

Center for Grapevine Biotechnology, The Darr College of Agriculture, Missouri State University, Springfield, MO 65897, U.S.A.

Abstract

Grapevines (*Vitis* spp.) host viruses belonging to 17 families. Virus-associated diseases are a constant challenge to grape production. Genetic resources for breeding virus-resistant grape cultivars are scarce. ‘Norton’ is a hybrid grape of North American *Vitis aestivalis* and is resistant to powdery mildew and downy mildew. In this study, we assessed resistance of ‘Norton’ to grapevine vein clearing virus (GVCV), which is prevalent in native, wild Vitaceae and in vineyards in the Midwest region of the U.S. We did not detect GVCV in ‘Norton’ as either the scion or the rootstock up to 3 years after it

was grafted with a GVCV-infected ‘Chardonnay’ grapevine. Upon sequencing of small RNAs, we were able to assemble the GVCV genome from virus small RNAs in GVCV-infected ‘Chardonnay’ scion or rootstock, but not from grafted ‘Norton’ scion and rootstock. This study unveils a new trait of ‘Norton’ that can be used in breeding GVCV-resistant grape cultivars, and to investigate genetic mechanisms of ‘Norton’ resistance to GVCV.

Keywords: ‘Norton’ virus, genetics, resistance

Grapevine (*Vitis* spp.) is host to nearly 70 viruses from 17 families (Martelli 2017). An individual grapevine can be infected with a single virus species, or simultaneously with multiple viruses across families. Virus-associated diseases reduce berry quality and shorten the grapevine’s lifespan in a vineyard. Perennial growth of grapevines in both native habitats and vineyards exposes them continually to potential virus infection. In native habitats, insect vectors are the main transmission routes of viruses, and incidence of viral infection increases with dynamic epidemics of insect populations. In commercial production, vegetative propagation is the major means of disseminating viruses (Rowhani et al. 2005). Incidence of virus-associated diseases in vineyards increases with demand for propagating more grapevines in the expansion of viticulture.

In recent years, new viruses have emerged in vineyards and in wild grapevines (Maliogka et al. 2015; Perry et al. 2016; Qiu and Schoelz 2017; Sudarshana et al. 2015). They are bringing new and constant challenges to grape production, and grapevine vein clearing virus (GVCV) is one of these emerging challenges (Cieniewicz et al. 2020). GVCV belongs to the genus *Badnavirus* in the family *Caulimoviridae* (Zhang et al. 2011). Infection by GVCV stunts grapevine growth over a period of 3 to 7 years, deforms berry clusters, and even kills susceptible vines. The virus spreads among grape cultivars (Guo et al. 2014) as well as in wild *Vitis* species (Beach et al. 2017) and *Ampelopsis cordata* plants of the Vitaceae family (Petersen et al. 2019). Incidence of GVCV in wild *Vitis* species and *A. cordata* vines averages 10% and 34%, respectively. The grape aphid (*Aphis illinoensis*) is a reported insect vector (Petersen et al. 2019). Natural reservoirs of GVCV and grape aphids as transmission vectors provide multiple opportunities for virus infection in vineyards. Discovering GVCV-resistant grape cultivars would help alleviate losses of

vineyards and provide germplasm for breeding virus-resistant grapevines.

Genetic sources of natural resistance to grapevine viruses are scarce. One study reported that a wild Middle Eastern *V. vinifera*, a *V. rotundifolia* cultivar, and a hybrid grape of *V. vinifera* and *V. rotundifolia* were resistant to grapevine fanleaf virus (GFLV) (Walker et al. 1985). After screening 223 *Vitis* species and interspecific hybrids for natural resistance to GFLV, arabis mosaic virus (ArMV), grapevine leafroll-associated virus 1 (GLRaV-1) and GLRaV-3, Lahogue and Boulard did not find a single virus-resistant accession (Lahogue and Boulard 1996). No sources of natural resistance in *Vitis* germplasm or rootstocks of cultivated grapes have been found to grapevine viruses that are associated with leafroll disease, rugose wood disease complex, or fleck disease complex (Oliver and Fuchs 2011). A collection of wild and cultivated *Vitis* and *Muscadinia* species were screened for virus resistance, but no germplasm was proven to be resistant to GFLV or GLRaVs (Oliver and Fuchs 2011). Therefore, continuous screening of *Vitis* germplasm for resistance against viruses is much needed (Oliver and Fuchs 2011). In our previous study (Guo et al. 2014), we reported that the hybrid grape ‘Chambourcin’ is resistant to GVCV. In this study, we assessed the resistance of the North American grape ‘Norton’ to GVCV.

‘Norton’ has been a major hybrid grape for red wine production in the midwestern to southeastern regions of the U.S. for over 180 years (Ambers 2013). ‘Norton’ berries have a high anthocyanin content and unique profiles of anthocyanins and proanthocyanidins (Ali et al. 2011). ‘Norton’ is resistant to major fungal pathogens including powdery mildew (*Erysiphe necator*) (Fung et al. 2008) and downy mildew (*Plasmopara viticola*) (Sapkota et al. 2018). High contents of salicylic acids and secondary metabolites are constitutively associated with high levels of ‘Norton’ disease resistance (Fung et al. 2008). However, whether ‘Norton’ is resistant to viruses has not yet been addressed.

To investigate if ‘Norton’ is resistant to GVCV, we inoculated ‘Norton’ grapevines with GVCV via graft-transmission. We performed wedge-grafting experiments using green cuttings of GVCV-infected ‘Chardonnay’ grapevines and GVCV-free ‘Norton’ grapevines. In one set of three graftings, GVCV-infected ‘Chardonnay’ grapevine was used as scion and ‘Norton’ grapevine as rootstock, and only one grafted vine survived for further assessment (Table 1). In the reciprocal grafting, ‘Norton’ grapevines served as scion and

[†]Corresponding author: W. P. Qiu; WenpingQiu@missouristate.edu

Funding: We thank the Missouri Wine and Grape Board for continuous support of our research program in the Center for Grapevine Biotechnology.

The author(s) declare no conflict of interest.

Accepted for publication 3 March 2020.

GVCV-infected 'Chardonnay' grapevines as rootstock and six grafted vines survived (Table 1). The grafting of 'Norton' as rootstock or scion ensures systemic movement of GVCV from shoots to roots or vice versa. DNA was extracted from young leaves on the upper part of each branch. Polymerase chain reaction (PCR) was used to detect GVCV as previously described (Beach et al. 2017; Petersen et al. 2019). Detection of GVCV was carried out on grafted vines that were grown in the greenhouse periodically over 3 years after grafting (Table 1). GVCV was detected in both 'Chardonnay' (not represented in Fig. 1) and 'Cabernet Sauvignon' scions that were grafted onto GVCV-infected 'Chardonnay' rootstock (Fig. 1, lane 1 and 2). In contrast, GVCV was not detected in 'Norton,' either as scion (Fig. 1, lane 3 to 8) or rootstock (Fig. 1, lane 9 and 10). In the first case, it shows that GVCV did not move downward into the 'Norton' rootstock from the infected scion. In the second case, it indicates that GVCV did not move upward into the 'Norton' scion from the infected rootstock. These results suggest that either GVCV cannot move through the vascular tissues via the graft union or cannot replicate in the grafted

Fig. 1. Detection of grapevine vein clearing virus (GVCV) in grafted grapevines by PCR using two sets of GVCV-specific primers and one set of 16S rRNA primers. M: markers of DNA molecule size in base pairs. Lanes 1 and 2: 'Cabernet Sauvignon' scion (lane 1) on GVCV-infected 'Chardonnay' rootstock (lane 2) 1 year after grafting. Lanes 3 and 4: 'Norton' scion (lane 3) on GVCV-infected 'Chardonnay' rootstock (lane 4) 3 years after grafting. Lanes 5 and 6: 'Norton' scion (lane 5) on GVCV-infected 'Chardonnay' rootstock (lane 6) 1 year after grafting. Lanes 7 and 8: 'Norton' scion (lane 7) on GVCV-infected 'Chardonnay' rootstock (lane 8) 1 year after grafting. Lanes 9 and 10: GVCV-infected 'Chardonnay' scion (lane 9) on 'Norton' rootstock (lane 10) 3 years after grafting. Lanes 11 and 12: 'Vignoles' scion (lane 11) on GVCV-infected 'Chardonnay' rootstock (lane 12) 3 years after grafting, which was used as additional cultivar for resistance assessment. P: GVCV-infected 'Chardonnay' 'LBC0903' was used as positive control; N: PCR without DNA template as negative control.

'Norton' grapevine. Although inoculation of a virus by graft-transmission cannot control the amount of virus inoculum (Oliver and Fuchs 2011) and the viral titer is too high for accurately assessing natural resistance (Laimer et al. 2009), graft-transmission ensures the presence of abundant viral inoculum. The PCR assay for GVCV was repeated periodically for 3 years after grafting. Absence of GVCV in 'Norton' as scion or rootstock clearly indicates that 'Norton' is resistant to GVCV up to 3 years after inoculation.

The above experiments were done under greenhouse conditions. We further evaluated 'Norton' resistance to GVCV in vineyards where both viral inoculum (reservoir) and an insect vector were present. The results from evaluation of 'Norton' resistance to GVCV in vineyards would reflect a real-world status of viruses and 'Norton' performance in commercial vineyards. Fortunately, a vineyard was available for us to perform this evaluation. In 2007, we planted virus-tested grape hybrids in a foundation vineyard at the Missouri State Fruit Experiment Station, Mountain Grove, Missouri, U.S.A. After we discovered that GVCV was associated with grapevine vein-clearing and vine decline disease (Zhang et al. 2011), we conducted a survey of GVCV in 86 'Chardonnay' vines in 2014, 2016, and 2018. GVCV was detected in 13%, 4%, and 3% of 'Chardonnay,' respectively. Decreased incidence is a result of removing GVCV-infected vines as soon as they were discovered. It was clear that GVCV had spread in the foundation vineyard since 2007. 'Vidal blanc' was infected with GVCV at a 30% infection rate in 2016, and thus removed from the foundation vineyard. In 2018, we conducted a survey of GVCV in all 74 'Norton' vines and found no incidence of this virus. 'Norton' and 'Chardonnay' vines were planted in two rows each, 32 feet (9.8 m) apart, in the same foundation vineyard in 2007. It is clear that 'Norton' had not acquired GVCV in 12 years, although both inoculum source and insect vector had been present in the vineyard.

Small RNAs have been shown to transport through the graft union and systemically move through the entire plant (Melnik et al. 2011; Molnar et al. 2010). Viral small RNAs (vsRNAs) of GVCV have been detected in susceptible grapevines (Howard and Qiu 2017). To verify the above results and also investigate possible mechanisms for viral resistance in 'Norton,' we began by determining if we could detect GVCV vsRNAs in the graft-inoculated 'Norton.' Total RNA was sent to Admera Health (126 Corporate Blvd, South Plainfield, NJ 07080) for acquiring sequences of small RNAs. The cDNA library construction and RNA size selection were carried out using NEBNext Multiplex Small RNA Library Prep Set for Illumina sequencing (New England Biolabs Inc, Ipswich, MA, U.S.A.)

Table 1. Assessment of 'Norton' resistance via graft-transmission to grapevine vein clearing virus (GVCV) by polymerase chain reaction (PCR)

Scion	Rootstock	Vines grafted	Vines analyzed	Age of grafted vines in years	Detection of GVCV	
					Scion	Rootstock
Norton	GVCV-Chardonnay ^a	6	6	1–3	–	+
GVCV-Chardonnay	Norton	3	1	3	+	–
Cabernet Sauvignon	GVCV-Chardonnay	3	1	1	+	+
Chardonnay	GVCV-Chardonnay	3	1	1	+	+
GVCV-Chardonnay	Cabernet Sauvignon	3	1	3	+	+

^a GVCV-infected 'Chardonnay' grapevine was used as virus inoculum.

Table 2. Assessment of 'Norton' resistance to grapevine vein clearing virus (GVCV) by RNAseq of small RNAs in GVCV-grafted 'Norton' versus GVCV-grafted 'Cabernet Sauvignon' 3 years after graft-transmission

Scion/Rootstock	vsRNA profiles by RNAseq					
	Reads mapped to GVCV in RPM ^a	Percentage of genome assembled	21 nt	22 nt	24 nt	>24 nt
Norton/	4.7	0	51	8	2	0
GVCV-Chardonnay	33,029.0	100	403,627	93,338	15,303	5,595
GVCV-Chardonnay/	3,398.8	99.59	37,310	13,414	1,674	1,108
Norton	9.4	0	100	24	5	1
GVCV-Chardonnay/	8,237	100	79,226	8,259	1,313	2,715
Cabernet Sauvignon	40,713	100	319,114	83,238	13,476	12,332

^a RPM: reads per million.

following the manufacturer's protocol. The library was sequenced on the Illumina HiSeq for a length of 150 nt. After primer sequence removal with Cutadapt 1.9.1, the reads were checked for quality using a cutoff of Phred score 20 and a cutoff length of 17 nt (NGS QC V2.3.3). The subsequent procedures of aligning reads to the GVCV reference genome, calculating reads per million, and assembling the GVCV genome from vsRNAs have been described previously (Howard and Qiu 2017). We were able to assemble the GVCV genome with 100% and 99.6% coverage, respectively, from vsRNAs in the infected 'Chardonnay' grapevines as rootstock or scion (Table 2). The whole GVCV genome with 100% coverage was also assembled in the GVCV-grafted 'Cabernet Sauvignon.' The 21-nt small RNAs are predominant among the vsRNAs in all samples, revealing a characteristic feature of the RNA silencing pathway in grapevines. Only 4.7 and 9.4 reads per million (RPM) were mapped to the GVCV genome in the grafted 'Norton' scion and rootstock in comparison with the 33,029 and 3,398 RPM in GVCV-infected rootstock and scion (Table 2). These results show that only a very small number of GVCV-specific vsRNAs may have been transferred to 'Norton' rootstock or scion through the graft union. We will need to investigate if this small number of vsRNAs would play a role in 'Norton's' resistance to GVCV, or they are mere background of the RNA-seq technology.

A plant virus replicates in a single cell, moves locally to the neighboring cells, and then systemically through vascular tissues to establish infection in the entire plant. In response, the plant resists a virus via gene silencing, R-gene mediated innate immunity, or the blocking of local and systemic movement. Some plants may not possess host factors that are essential for viral replication and movement (Kang et al. 2005). In a previous study, we compiled GVCV-derived small RNAs (Howard and Qiu 2017). We searched these vsRNAs against the sequenced draft genome of 'Norton' and did not find homologous regions on the 'Norton' genome that align with GVCV-derived vsRNAs. This suggests that 'Norton' may not encode miRNAs that participate in the active defense pathways of degrading GVCV-derived RNAs. Inability of GVCV to move through the graft union suggests that 'Norton' as scion or rootstock cannot accept GVCV into vascular tissues, either blocking GVCV movement or lacking host factors assisting GVCV movement or replication.

Genetic background plays a significant role in plants' resistance to viruses (Gallois et al. 2018). *V. aestivalis* is native to the Midwest region of the U.S. where GVCV is present at 34% incidence in wild native *A. cordata* plants (Petersen et al. 2019) and in wild *Vitis* plants (Beach et al. 2017). Coevolution of *V. aestivalis* and GVCV selects for resistant wild *V. aestivalis* accessions. 'Norton' genetic background derives mainly from wild *V. aestivalis* (Ambers 2013; Sawler et al. 2013). Multiple genes from wild *V. aestivalis* may contribute to form innate immunity to GVCV. Host factors also contribute to each phase of a virus's life cycle: translation, replication, transcription, movement, and virion assembly (Garcia-Ruiz 2018), and thus are referred to as susceptibility genes. The lack of these host factors forms another line of resistance in that the virus is unable to complete the infection cycle in a host that does not possess these cellular factors (Garcia-Ruiz 2018). One possible scenario is that 'Norton' may not encode the genes that are required for GVCV replication and movement. Therefore, genetic and molecular mechanisms underlying 'Norton' resistance to GVCV merit further investigation.

Conclusions

'Norton' is found to be resistant to GVCV by graft transmission. The conclusion is further supported by absence of GVCV in 'Norton' grapevines in vineyards. This unveils a new trait of 'Norton' grapevine. Therefore, 'Norton' can be used as a parent in breeding GVCV-resistant varieties. Given that virus-resistant *Vitis* germplasm is scarce, it is worthwhile to assess 'Norton' resistance to grapevine viruses other than GVCV. Further study on the genetic and molecular mechanisms underlying 'Norton' resistance to GVCV will provide novel insights on understanding a woody perennial plant's resistance to a virus and interconnectedness of 'Norton' resistance to multiple pathogens.

Acknowledgments

We are thankful to those undergraduate and graduate students over the last five years who have contributed to this project.

Literature Cited

- Ali, M., Howard, S., Chen, S., Wang, Y., Yu, O., Kovacs, L., and Qiu, W. 2011. Berry skin development in Norton grape: Distinct patterns of transcriptional regulation and flavonoid biosynthesis. *BMC Plant Biol.* 11:7.
- Ambers, C. P. 2013. A historical hypothesis on the origin of the Norton grape. *J. Wine Res.* 24:85-95.
- Beach, S., Kovens, M., Hubbert, L., Honesty, S., Guo, Q., Pap, D., Dai, R., Kovacs, L., and Qiu, W. 2017. Genetic and phenotypic characterization of *Grapevine vein clearing virus* from wild *Vitis rupestris*. *Phytopathology* 107:138-144.
- Cieniewicz, E., Qiu, W., Saldarelli, P., and Fuchs, M. 2020. Believing is seeing: lessons from emerging viruses in grapevine. *J. Plant Pathol.* <https://doi.org/10.1007/s42161-019-00484-3>
- Fung, R. W. M., Gonzalo, M., Fekete, C., Kovacs, L. G., He, Y., Marsh, E., McIntyre, L. M., Schachtman, D. P., and Qiu, W. P. 2008. Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. *Plant Physiol.* 146:236-249.
- Gallois, J.-L., Moury, B., and German-Retana, S. 2018. Role of the genetic background in resistance to plant viruses. *Int. J. Mol. Sci.* 19:2856.
- Garcia-Ruiz, H. 2018. Susceptibility genes to plant viruses. *Viruses* 10:484.
- Guo, Q., Honesty, S., Xu, M. L., Zhang, Y., Schoelz, J. E., and Qiu, W. P. 2014. Genetic diversity, tissue and host specificity of *Grapevine vein clearing virus*. *Phytopathology* 104:539-547.
- Howard, S., and Qiu, W. 2017. Viral small RNAs reveal the genomic variations of three grapevine vein clearing virus quasispecies populations. *Virus Res.* 229:24-27.
- Kang, B. C., Yeam, I., and Jahn, M. M. 2005. Genetics of plant virus resistance. *Ann. Rev. Phytopathol.* 43:581-621.
- Lahogue, F., and Boulard, G. 1996. Recherche de genes de resistance naturelle a deux viroses de la vigne: le court-noué et l'enroulement. *Vitis* 35:43-48.
- Laimer, M., Lemaire, O., Herrbach, E., Goldschmidt, V., Minafra, A., Bianco, P., and Wetzel, T. 2009. Resistance to viruses, phytoplasmas and their vectors in the grapevine in Europe: A review. *J. Plant Pathol.* 91:7-23.
- Maliogka, V. I., Olmos, A., Pappi, P. G., Lotos, L., Efthimiou, K., Grammatikaki, G., Candresse, T., Katis, N. I., and Avgelis, A. D. 2015. A novel grapevine badnavirus is associated with the Roditis leaf discoloration disease. *Virus Res.* 203:47-55.
- Martelli, G. P. 2017. An overview on grapevine viruses, viroids, and the diseases they cause. Pages 31-46 in: *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. B. Meng, G. P. Martelli, D. A. Golino, and M. Fuchs, eds. Springer International Publishing, Cham, Switzerland.
- Melnyk, C. W., Molnar, A., and Baulcombe, D. C. 2011. Intercellular and systemic movement of RNA silencing signals. *EMBO J.* 30:3553-3563.
- Molnar, A., Melnyk, C. W., Bassett, A., Hardcastle, T. J., Dunn, R., and Baulcombe, D. C. 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328:872-875.
- Oliver, J. E., and Fuchs, M. 2011. Tolerance and resistance to viruses and their vectors in *Vitis* sp.: A virologist's perspective of the literature. *Am. J. Enol. Vitic.* 62:438-451.
- Perry, K. L., McLane, H., Hyder, M. Z., Dangi, G. S., Thompson, J. R., and Fuchs, M. F. 2016. Grapevine red blotch-associated virus is present in free-living *Vitis* spp. proximal to cultivated grapevines. *Phytopathology* 106:663-670.
- Petersen, S. M., Keith, C., Austin, K., Howard, S., Su, L., and Qiu, W. 2019. A natural reservoir and transmission vector of Grapevine vein clearing virus. *Plant Dis.* 103:571-577.
- Qiu, W., and Schoelz, J. 2017. Grapevine vein clearing virus: Diagnostics, genome, genetic diversity, and management. Pages 315-330 in: *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. B. Meng, G. P. Martelli, D. A. Golino, and M. Fuchs, eds. Springer International Publishing, Cham, Switzerland.
- Rowhani, A., Uyemoto, J. K., Golino, D. A., and Martelli, G. P. 2005. Pathogen testing and certification of *Vitis* and *Prunus* species. *Annu. Rev. Phytopathol.* 43:261-278.
- Sapkota, S., Chen, L.-L., Yang, S., Hyma, K. E., Cadle-Davidson, L., and Hwang, C.-F. 2018. Construction of a high-density linkage map and QTL detection of downy mildew resistance in *Vitis aestivalis*-derived 'Norton'. *Theor. Appl. Genet.* 132:137-147.
- Sawler, J., Reisch, B., Aradhya, M. K., Prins, B., Zhong, G.-Y., Schwaninger, H., Simon, C., Buckler, E., and Myles, S. 2013. Genomics assisted ancestry deconvolution in grape. *PLoS One* 8:e80791.
- Sudarshana, M. R., Perry, K. L., and Fuchs, M. F. 2015. Grapevine red blotch-associated virus, an emerging threat to the grapevine industry. *Phytopathology* 105:1026-1032.
- Walker, M. A., Meredith, C. P., and Goheen, A. C. 1985. Sources of resistance to grapevine fanleaf virus (GFV) in *Vitis* species. *Vitis* 24:218-228.
- Zhang, Y., Singh, K., Kaur, R., and Qiu, W. 2011. Association of a novel DNA virus with the grapevine vein-clearing and vine decline syndrome. *Phytopathology* 101:1081-1090.