

First Report of Grapevine Leafroll-Associated Virus-3 Infecting *Vitis vinifera* L. Grape in Alabama

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Grapevine leafroll-associated virus-3 (GLRaV-3) is considered the most predominant cause of grapevine leafroll disease (GLD), one of the most destructive viral diseases affecting grapevines and wine production worldwide (Maree et al. 2013). GLRaV-3 is a positive-sense, single-stranded RNA (+ssRNA) virus in the family *Closteroviridae*, genus *Ampelovirus*, species *tritis* (Martelli et al., 2012; Maree et al., 2013). Grape plants (cultivar ‘Errante Noir’) were planted at the Chilton Research and Extension Center (CREC), Chilton County, Alabama, in the spring of 2024. In November 2024, a plant exhibited symptoms of dark-purplish red veins with cupping of the leaf margins, and in July 2025, symptoms of reddish-brown blotches randomly distributed on the foliage appeared (Supplemental Figure 1: A: I-II). To investigate if a pathogen caused this, a sample was sent to Agdia, Inc. (Elkhart, IN, USA), where tissues were subjected to a pathogen screen, including alfalfa mosaic virus (AMV), arabis mosaic virus (ArMV), grapevine fanleaf virus (GFLV), *Phytophthora* (Phyt), peach rosette mosaic virus (PRMV), strawberry latent ringspot virus (SLRSV), tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV), GLRaV-3, grapevine pinot gris virus (GPGV), grapevine red blotch-associated virus (GRBaV), and *Xylella fastidiosa* (Xf). The sample tested negative for all except for GLRaV-3. To confirm GLRaV-3, RT-PCR was used. Total RNA was extracted from 0.1 g petiole tissue from three subsamples using the RNeasy Plant Mini Kit (Qiagen), producing three separate RNA samples. The cDNA was synthesized using SuperScript IV Reverse Transcriptase (Invitrogen), and Platinum Taq DNA Polymerase (Invitrogen) was used to amplify the heat shock protein (HSP70) and the coat protein (CP) using specific primers (Thompson et al. 2019). PCR products were checked on the TapeStation using the D1000 ScreenTape kit (Agilent), and amplicons corresponding to 600 bp (HSP70) and 280 bp (CP) were detected (Supplemental Figure 1: B). Sanger sequencing was conducted at Azenta Life Sciences in both directions. The manufacturer’s instructions were followed in all used kits. Sequences were analyzed using BLASTn (Altschul et al. 1990) to confirm that all three sequences from each gene match GLRaV-3. Sequences were submitted to GenBank (accessions: PX123059-64), and an isolate name was given (GLRaV-3_Chilton-AL.1.1-3). The closest BLAST hit is GLRaV3-8415B (KY073324.1) with 100% (HSP70), 98.24% (CP) nucleotide similarities (Supplemental Table 1). The CP sequences of GLRaV-3_Chilton-AL.1.1-3 were compared to 139 isolates of GLRaV-3 from GenBank (Supplemental Table 1) to construct a maximum likelihood tree using methods described by Shehata et al. (2025), with a modification where IQ-TREE was used to construct the tree with 1000 replicates (Nguyen et al. 2015). This tree indicated that GLRaV-3_Chilton-AL.1.1-3 is within group XII, with two isolates from Canada

(Supplemental Figure 1: C). Other grape plants planted along with the infected plant at the Chilton Co. site (n=15) were also tested for GLRaV-3 as described above, and all tested negative. This constitutes the first report of GLRaV-3 in Alabama, highlighting the importance of purchasing clean material to prevent introduction of the disease in vineyards, a crop with a total impact of \$1.5 billion in Alabama (Good, T. 2023).

Keywords

Grapevine leafroll-associated virus-3, isothermal amplification, RT-PCR, and grape production.

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Conflict of interest

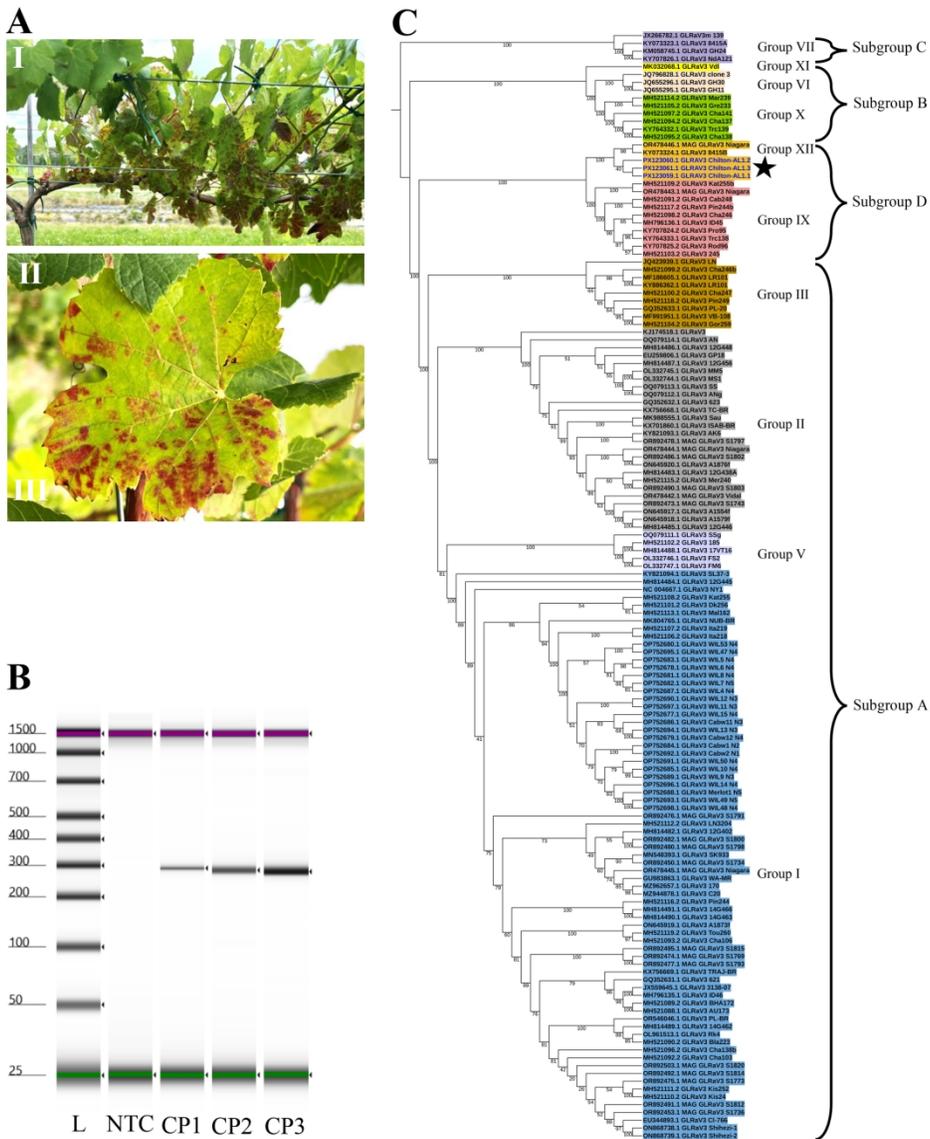
The author(s) declare no conflict of interest.

References

- Altschul, S. F., et al. 1990. *J Mol Biol.* 215:403-10. [http://doi:10.1016/S0022-2836\(05\)80360-2](http://doi:10.1016/S0022-2836(05)80360-2)
- Good, T., 2023. Wine America. “State Profile: Alabama Wine Country.” *Wine America News*, 5 Jan. 2023. <https://wineamerica.org/news/state-profile-alabama-wine-country/>
- Maree, H. J., et al. 2013. *Front Microbiol.* 4:82. <http://doi:10.3389/fmicb.2013.00082>
- Martelli, G., et al. 2012. *J Plant Pathol.* 94:7-19. <http://www.jstor.org/stable/45156004>
- Nguyen, L. T., et al. 2015. *Mol Biol Evol.* 32:268-74. <http://doi:10.1093/molbev/msu300>
- Shehata, A. H. A., et al. 2025. *Virology.* 604, 110450. <https://doi.org/10.1016/j.virol.2025.110450>
- Thompson, B. D., et al. 2019. *Plant Dis.* 103:509-18. <https://doi.org/10.1094/PDIS-08-18-1303-RE>

Figure legend:

Supplemental Figure 1: A: Photograph of the leaf tissue between the veins turned dark purplish-red with cupping of the leaf margins observed in November 2024 and reddish-brown blotch symptoms observed on the grapevine plants in the summer of 2025 at the Chilton Research and Extension Center (CREC), Chilton County, Alabama. I and II: Images taken in June-July 2025. B: The gel from the TapeStation, which indicates the detection of a single band of 280 bp for the coat protein (CP) from the three samples. Lanes (left to right): electronic ladder, negative control, coat protein samples CP1, CP2, and CP3. C: The phylogenetic tree analysis constructed with IQ-TREE and visualized using iTOL of 139 sequences of GLRaV3 obtained from GenBank, in addition to the three sequences obtained in this study (GLRaV-3_Chilton-AL.1.1-3), marked with a black asterisk, clustering in Group XII in Subgroup D.



Supplemental Figure 1: A: Photograph of the leaf tissue between the veins turned dark purplish-red with cupping of the leaf margins observed in November 2024 and reddish-brown blotch symptoms observed on the grapevine plants in the summer of 2025 at the Chilton Research and Extension Center (CREC), Chilton County, Alabama. I and II: Images taken in June-July 2025. B: The gel from the TapeStation, which indicates the detection of a single band of 280 bp for the coat protein (CP) from the three samples. Lanes (left to right): electronic ladder, negative control, coat protein samples CP1, CP2, and CP3. C: The phylogenetic tree analysis constructed with IQ-TREE and visualized using iTOL of 139 sequences of GLRaV3 obtained from GenBank, in addition to the three sequences obtained in this study (GLRaV-3_Chilton-AL.1.1-3), marked with a black asterisk, clustering in Group XII in Subgroup D.

203x238mm (300 x 300 DPI)

Supplemental Table 1. Assigned GenBank accession numbers for grapevine leafroll-associated virus 3 (GLRaV-3) heat shock protein 70 (HSP70) and coat protein (CP) sequences generated from Alabama isolate in this study, along with GenBank accessions of 139 previously published GLRaV-3 isolates included in the maximum-likelihood phylogenetic analysis. For all isolate, the table lists the isolate name and gene. For each sequence from Alabama isolate, the table indicates nucleotide length, BLAST closest hit, and nucleotide similarity (%).

No	Accession number	Isolate name	Gene	Length, BLAST closest hit, and nucleotide similarity % for AL isolate of GLRaV3.
1	PX123062.1	GLRaV3 Chilton-AL1.1	HSP70	548 bp, KY073324.1, 100%
2	PX123063.1	GLRaV3 Chilton-AL1.2	HSP70	548 bp, KY073324.1, 100%
3	PX123064.1	GLRaV3 Chilton-AL1.3	HSP70	548 bp, KY073324.1, 100%
4	PX123059.1	GLRaV3 Chilton-AL1.1	CP	285 bp, KY073324.1, 98.24%
5	PX123060.1	GLRaV3 Chilton-AL1.2	CP	285 bp, KY073324.1, 98.24%
6	PX123061.1	GLRaV3 Chilton-AL1.3	CP	285 bp, KY073324.1, 98.24%
7	JX266782.1	GLRaV3m 139	CP	
8	KY073323.1	GLRaV3 8415A	CP	
9	KM058745.1	GLRaV3 GH24	CP	
10	KY707826.1	GLRaV3 NdA121	CP	
11	MK032068.1	GLRaV3 Vdl	CP	
12	JQ796828.1	GLRaV3 clone 3	CP	
13	JQ655296.1	GLRaV3 GH30	CP	
14	JQ655295.1	GLRaV3 GH11	CP	
15	MH521114.2	GLRaV3 Mar239	CP	
16	MH521105.2	GLRaV3 Gre233	CP	
17	MH521097.2	GLRaV3 Cha141	CP	
18	MH521094.2	GLRaV3 Cha137	CP	
19	KY764332.1	GLRaV3 Trc139	CP	
20	MH521095.2	GLRaV3 Cha138	CP	
21	OR478446.1	MAG GLRaV3 Niagara	CP	
22	KY073324.1	GLRaV3 8415B	CP	
23	MH521109.2	GLRaV3 Kat255b	CP	
24	OR478443.1	MAG GLRaV3 Niagara	CP	
25	MH521091.2	GLRaV3 Cab248	CP	
26	MH521117.2	GLRaV3 Pin244b	CP	
27	MH521098.2	GLRaV3 Cha246	CP	
28	MH796136.1	GLRaV3 ID45	CP	
29	KY707824.2	GLRaV3 Pro95	CP	
30	KY764333.1	GLRaV3 Trc138	CP	
31	KY707825.2	GLRaV3 Rod96	CP	
32	MH521103.2	GLRaV3 245	CP	
33	JQ423939.1	GLRaV3 LN	CP	
34	MH521099.2	GLRaV3 Cha246b	CP	
35	MF186605.1	GLRaV3 LR101	CP	
36	KY886362.1	GLRaV3 LR101	CP	

37	MH521100.2	GLRaV3 Cha247	CP
38	MH521118.2	GLRaV3 Pin249	CP
39	GQ352633.1	GLRaV3 PL-20	CP
40	MF991951.1	GLRaV3 VB-108	CP
41	MH521104.2	GLRaV3 Gor259	CP
42	KJ174518.1	GLRaV3	CP
43	OQ079114.1	GLRaV3 AN	CP
44	MH814486.1	GLRaV3 12G448	CP
45	EU259806.1	GLRaV3 GP18	CP
46	MH814487.1	GLRaV3 12G456	CP
47	OL332745.1	GLRaV3 MM5	CP
48	OL332744.1	GLRaV3 MS1	CP
49	OQ079113.1	GLRaV3 SS	CP
50	OQ079112.1	GLRaV3 ANg	CP
51	GQ352632.1	GLRaV3 623	CP
52	KX756668.1	GLRaV3 TC-BR	CP
53	MK988555.1	GLRaV3 Sau	CP
54	KX701860.1	GLRaV3 ISAB-BR	CP
55	KY821093.1	GLRaV3 AK6	CP
56	OR892478.1	MAG GLRaV3 S1797	CP
57	OR478444.1	MAG GLRaV3 Niagara	CP
58	OR892486.1	MAG GLRaV3 S1802	CP
59	ON645920.1	GLRaV3 A1876f	CP
60	MH814483.1	GLRaV3 12G438A	CP
61	MH521115.2	GLRaV3 Mer240	CP
62	OR892490.1	MAG GLRaV3 S1803	CP
63	OR478442.1	MAG GLRaV3 Vidal	CP
64	OR892473.1	MAG GLRaV3 S1743	CP
65	ON645917.1	GLRaV3 A1554f	CP
66	ON645918.1	GLRaV3 A1579f	CP
67	MH814485.1	GLRaV3 12G446	CP
68	OQ079111.1	GLRaV3 SSg	CP
69	MH521102.2	GLRaV3 185	CP
70	MH814488.1	GLRaV3 17VT16	CP
71	OL332746.1	GLRaV3 FS2	CP
72	OL332747.1	GLRaV3 FM6	CP
73	KY821094.1	GLRaV3 SL37-3	CP
74	MH814484.1	GLRaV3 12G445	CP
75	NC 004667.1	GLRaV3 NY1	CP
76	MH521108.2	GLRaV3 Kat255	CP
77	MH521101.2	GLRaV3 Dk256	CP
78	MH521113.1	GLRaV3 Mal162	CP
79	MK804765.1	GLRaV3 NUB-BR	CP
80	MH521107.2	GLRaV3 Ita219	CP
81	MH521106.2	GLRaV3 Ita218	CP
82	OP752680.1	GLRaV3 WIL53 N4	CP

83	OP752695.1	GLRaV3 WIL47 N4	CP
84	OP752683.1	GLRaV3 WIL5 N4	CP
85	OP752678.1	GLRaV3 WIL6 N4	CP
86	OP752681.1	GLRaV3 WIL8 N4	CP
87	OP752682.1	GLRaV3 WIL7 N5	CP
88	OP752687.1	GLRaV3 WIL4 N4	CP
89	OP752690.1	GLRaV3 WIL12 N3	CP
90	OP752697.1	GLRaV3 WIL11 N3	CP
91	OP752677.1	GLRaV3 WIL15 N4	CP
92	OP752686.1	GLRaV3 Cabw11 N3	CP
93	OP752694.1	GLRaV3 WIL13 N3	CP
94	OP752679.1	GLRaV3 Cabw12 N4	CP
95	OP752684.1	GLRaV3 Cabw1 N2	CP
96	OP752692.1	GLRaV3 Cabw2 N1	CP
97	OP752691.1	GLRaV3 WIL50 N4	CP
98	OP752685.1	GLRaV3 WIL10 N4	CP
99	OP752689.1	GLRaV3 WIL9 N3	CP
100	OP752696.1	GLRaV3 WIL14 N4	CP
101	OP752688.1	GLRaV3 Merlot1 N5	CP
102	OP752693.1	GLRaV3 WIL49 N5	CP
103	OP752698.1	GLRaV3 WIL48 N4	CP
104	OR892476.1	MAG GLRaV3 S1791	CP
105	MH521112.2	GLRaV3 LN3204	CP
106	MH814482.1	GLRaV3 12G402	CP
107	OR892482.1	MAG GLRaV3 S1800	CP
108	OR892480.1	MAG GLRaV3 S1798	CP
109	MN548393.1	GLRaV3 SK933	CP
110	OR892450.1	MAG GLRaV3 S1734	CP
111	OR478445.1	MAG GLRaV3 Niagara	CP
112	GU983863.1	GLRaV3 WA-MR	CP
113	MZ962657.1	GLRaV3 170	CP
114	MZ944878.1	GLRaV3 C20	CP
115	MH521116.2	GLRaV3 Pin244	CP
116	MH814491.1	GLRaV3 14G466	CP
117	MH814490.1	GLRaV3 14G463	CP
118	ON645919.1	GLRaV3 A1873f	CP
119	MH521119.2	GLRaV3 Tou260	CP
120	MH521093.2	GLRaV3 Cha106	CP
121	OR892495.1	MAG GLRaV3 S1815	CP
122	OR892474.1	MAG GLRaV3 S1769	CP
123	OR892477.1	MAG GLRaV3 S1793	CP
124	KX756669.1	GLRaV3 TRAJ-BR	CP
125	GQ352631.1	GLRaV3 621	CP
126	JX559645.1	GLRaV3 3138-07	CP
127	MH796135.1	GLRaV3 ID46	CP
128	MH521089.2	GLRaV3 BHA172	CP

129	MH521088.1	GLRaV3 AU173	CP
130	OR546046.1	GLRaV3 PL-BR	CP
131	MH814489.1	GLRaV3 14G462	CP
132	OL961513.1	GLRaV3 Rk4	CP
133	MH521090.2	GLRaV3 Bla223	CP
134	MH521096.2	GLRaV3 Cha138b	CP
135	MH521092.2	GLRaV3 Cha103	CP
136	OR892503.1	MAG GLRaV3 S1820	CP
137	OR892492.1	MAG GLRaV3 S1814	CP
138	OR892475.1	MAG GLRaV3 S1773	CP
139	MH521111.2	GLRaV3 Kis252	CP
140	MH521110.2	GLRaV3 Kis24	CP
141	OR892491.1	MAG GLRaV3 S1812	CP
142	OR892453.1	MAG GLRaV3 S1736	CP
142	EU344893.1	GLRaV3 CI-766	CP
144	ON868738.1	GLRaV3 Shihezi-1	CP
145	ON868739.1	GLRaV3 Shihezi-2	CP
