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First Report of Hoya tobamovirus 2 in *Hoya* Species in Australia

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Hoya tobamovirus 2 (HoTV-2; syn. Hoya necrotic spot virus, genus *Tobamovirus*, family Virgaviridae) infects ornamental *Hoya* plants and is recorded from Germany and South Korea (Gaafar et al. 2020; Kim and Jeong 2024). In 2022, one plant each of the *Hoya* hybrid cv. Sunrise and *Hoya carnosa* cv. Krinkle, with chlorosis and necrotic patches on their leaves and infected with impatiens necrotic spot virus (INSV; species *Orthotospovirus impatiensnecromaculae*, genus *Othotospovirus*, family Tospoviridae), were collected from a commercial nursery in Victoria, Australia. Total RNA was extracted from each sample using an RNeasy Plant Mini kit (Qiagen, Australia). Libraries were prepared with the NEBNext UltraII RNA Library Prep Kit (New England BioLabs, USA). Sequencing on a Novaseq 6000 instrument (Illumina, USA) resulted in 3,238,966 (cv. Sunrise) and 5,148,862 (cv. Krinkle) raw reads and trimming and filtering using fastp version 0.20.0 (Chen et al. 2018) resulted in 2,181,218 and 3,284,930 reads, respectively. The trimmed reads were de novo assembled using SPAdes version 3.14.1 (Prjibelski et al. 2020). Comparison of the assembled contigs to sequences in GenBank indicated the presence of HoTV-2 and INSV in both samples. The longest HoTV-2 contig (6409 nt) from *Hoya* hybrid cv. Sunrise had > 99% nucleotide identity with other HoTV-2 isolates (GenBank Accession Nos. OR876400.1, LC807720.1, MN961200.1 and PP701469.1). A total of 1437.8 reads re-mapped to the 6409 nt HoTV-2 contig from *Hoya* hybrid cv. Sunrise with a sequence depth of 1437.8X. PrimalScheme (Quick et al. 2017) was used to design 12 pairs of primers (Table 1), based on the sequence of isolate JKI_19-461-466 (OR876400.1) then RT-PCR and Sanger sequencing of overlapping genome regions confirmed the detection and genome arrangement of HoTV-2 in *Hoya* hybrid cv. Sunrise (HoTV-2 Vic; PV929820). HoTV-2 Vic had 99.92% and 99.84% nucleotide identity to isolate JKI_19-461-466 and the 5700 nt contig from *H. carnosa* cv. Krinkle, respectively.

Samples from 50 asymptomatic plants of ornamental and native *Hoya* spp. were collected from three Australian states (New South Wales, $n = 6$; Queensland, $n = 40$; Victoria, $n = 4$). HoTV-2 was detected in 30/50 samples, all from Queensland, by RT-PCR using primers HoTV-2 F9 and HoTV-2 R9 (Table 1). The sequenced amplicons of nine samples had > 99.6% nucleotide similarity to HoTV-2 VIC. The positive samples represented 2 Australian native *Hoya* species, *H. australis* and *H. macgillivrayi*, and 15 non-native species, *H. arnottiana*, *H. carnosa*, *H. cinnamomifolia*, *H. compacta*, *H. cumingiana*, *H. finlaysonii*, *H. fungi*, *H. fuscomarginata*, *H. kerii*, *H. latifolia*, *H. mindorensis*, *H. multiflora*, *H. obovata*, *H. obscura* and *H. shepherdii*. RT-PCR confirmed transmission by mechanical inoculation of HoTV-2 Vic to two uninfected *H. globulosa* plants, which developed mild chlorotic mottling on older leaves compared to uninoculated plants, and two plants of *H. coronaria*, which remained symptomless (Figure 1). INSV was not detected in any inoculated or uninoculated plant by RT-PCR (Mumford et al. 1996). Further work is required to determine if HoTV-2 was the cause of chlorotic mottling in *H. globulosa* and if it has the potential to cause symptoms in other *Hoya* species and hybrids. This is the first report of HoTV-2 in Australia.

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TABLE 1 | Nucleotide sequence, annealing temperature and expected amplicon size of the primers that were used to confirm the detection and genome arrangement of a Hoya tobamovirus 2 isolate from Victoria, Australia.

Primer name	Orientation	Primer sequence (5′–3′)	Position on OR876400.1	Genome regions	Annealing temperature (°C)	Amplicon size (bp)
HoTV-2 F1	Forward	GGCACACATCACAAACACTTTCA	59–81	Replicase	60	772
HoTV-2 R1	Reverse	ACAACTTGTCCCCATCCTTGAC	828–807	Replicase		
HoTV-2 F2	Forward	GGGGCAGCACTTTTGAGGAAA	693–713	Replicase	60	825
HoTV-2 R2	Reverse	ATTTCTCCCACACGAGTTCTGC	1515–1494	Replicase		
HoTV-2 F3	Forward	GCTATGACCTTCTTCCTACAGACG	1407–1430	Replicase	60	781
HoTV-2 R3	Reverse	CGCATGTTTCCTTTCTTGAGCG	2185–2164	Replicase		
HoTV-2 F4	Forward	CTTGCATCCTGTTCGTTGCATG	2073–2094	Replicase	60	817
HoTV-2 R4	Reverse	ACGTAAGCCTTGTCACACAGAC	2887–2866	Replicase		
HoTV-2 F5	Forward	GTCAGGAAGACTTACAGCACACT	2784–2806	Replicase	60	773
HoTV-2 R5	Reverse	CGGGAGTGCCTTGTCATAGTAG	3554–3533	Replicase		
HoTV-2 F6	Forward	TACAGTGTGTCATCCGCTCCTA	3423–3444	Replicase	60	827
HoTV-2 R6	Reverse	GAGTTCCAAAACCTCCATCGCT	4247–4226	Replicase		
HoTV-2 F7	Forward	TGAGCAAGATTGACAGTTCGAGG	4135–4157	Replicase	60	786
HoTV-2 R7	Reverse	AGATTACGGAAAAGACGGTTATCAGA	4918–4893	Replicase		
HoTV-2 F8	Forward	GGTCAGTTGAATGATGCAATTGCT	4809–4832	Replicase	60	801
HoTV-2 R8	Reverse	ATTCTTCGGATGGTTCACAGGC	5607–5586	Movement protein		
HoTV-2 F9	Forward	GGTGAAGGTTCTGTCGACTTCG	5479–5500	Movement protein	60	810
HoTV-2 R9	Reverse	GTTATCGTACACACCACGTCAGT	6286–6264	Coat protein		
HoTV-2 F10	Forward	GTATTTTTGTTTACCTTACATTTACAC	1–27	5′ untranslated region	57	827
HoTV-2 R10	Reverse	CAACTTGTCCCCATCCTTGA	827–808	Replicase		
HoTV-2 F11	Forward	GATAACTGATGTTGTGCGTTTCC	5908–5930	Coat protein	59	495
HoTV-2 R11	Reverse	GGGATTCGAACCCCTGGAA	6401–6386	Coat protein		
HoTV-2 F12	Forward	GATAACTGATGTTGTGCGTTTCC	5908–5930	Coat protein	59	519
HoTV-2 R12	Reverse	TGGGCCCCTACCCG	6424–6412	Coat protein		



FIGURE 1 | The dorsal surface of Hoya tobamovirus 2-inoculated (A) and uninoculated (B) *Hoya globulosa* and inoculated (C) and uninoculated (D) *Hoya coronaria* plants. Chlorotic mottling symptoms were only observed on the inoculated *Hoya globulosa* plants.

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