First report of *Ageratum latent virus* in *Veronica* species and in New Zealand

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Abstract

A new strain of *Ageratum latent virus* (AGLV) was found in symptomless leaves of *Veronica bollonsii*, *V. brevifracemosa* and *V. dieffenbachii* - the native shrubs in New Zealand. The partial genome sequence of the AGLV from *V. bollonsii* has 85.9–89.1% amino acid similarity to the Australian isolate AGLV-1998. This is the first record of the AGLV in *Veronica* species and in New Zealand.

Keywords dsRNA-specific antibody · MiSeq · 5' RACE · Indigenous

Ageratum latent virus (AGLV) (Genus *Ilarvirus*, family *Bromoviridae*) has a tripartite, positive-strand RNA genome which encodes five proteins: the replication protein 1a on the RNA1, the RNA-dependent RNA polymerase (RdRp), the protein 2b on the RNA2, and the movement (MP) and coat (CP) proteins on the RNA3 (Bujarski et al. 2019). Previously known as a strain of tobacco streak virus (TSV-1998), *Ageratum latent virus* was shown to be a separate species in 2013 (Sharman and Thomas 2013). AGLV has been previously reported from plants of *Ageratum houstonianum* and *Gomphocarpus physocarpus* in eastern Australia (Sharman and Thomas 2013). Infected hosts were typically symptomless, and this virus has been noted for transmission via pollen and thrips (Bujarski et al. 2019).

Advanced sequencing methods have enabled the discovery of many new viruses in asymptomatic wild plants (Roossinck et al. 2015). In New Zealand, despite concerted efforts (Blouin

et al. 2016), little has been uncovered on the prevalence of viruses in native plants (Veerakone et al. 2015). *Veronica* (Syn *Hebe*; Plantaginaceae) is the largest plant genus in New Zealand, comprising of more than 150 species (136 indigenous (Schönberger et al. 2020)). Exotic species *Veronica persica* has previously been described as a host of cucumber mosaic virus, watermelon mosaic virus 2, and turnip yellows virus (Fletcher et al. 1999; Fletcher 1989; Kyriakou 1984; Kyriakou et al. 1983).

We used a two-step generic approach to optimise the discovery of virus sequences by analysing both double-stranded RNA (dsRNA) and total RNA extracts from four native New Zealand plants. All plants used in this study were cultivated outside as part of the landscape of the Manaaki Whenua Landcare Research campus, Lincoln, New Zealand. Symptomless leaves were collected and dried in plastic bags using silica gel. Total RNA and dsRNA were extracted from four bushy plants - *Brachyglottis F. compacta*, *V. bollonsii*, *Myrtus bullata* and *Leptospermum scoparium* in triplicates using PureLink® RNA Mini Kit (ambion) and Double-RNA Viral dsRNA Extraction Mini Kit (iNtRON), as per the manufacturer's recommendations, eluted in 200 µL and 40 µL of water, and pooled (600 µL and 120 µL, respectively). In order to specifically target dsRNA, the 600 µL of total RNA was enriched using 0.2 ml 3G1 anti-dsRNA IgM antibody hybridoma supernatant (O'Brien et al. 2015) following the pull-down assay for recovery of dsRNA protocol of Blouin et al. (2016) and resuspended in 15 µL of water. Complementary DNA was synthesised using the N6 primer (CCTTCGGA TCCTCCNNNNN) as in Roossinck et al. (2010), and PCR was performed using the acgcgCTTCGGATCCTCC

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Table 1 Ageratum latent virus specific primers used in the molecular amplification (PCR and 5'-RACE)

Primer	Sequence 5'-3'	Amplicon (bp)	Target
RNA1-39F RNA1-408R	CCGAGAAGATCATTGACCTCG TCAACATTGAGTAAAGGTATATGACC A	370	Replicase protein 1a
RNA2-36F RNA2-356R	CAGATGGATCTGGTCTAACCTGAA ACGTTGAAAATGTCAGTCACATCC	321	RdRp
RNA3-9F RNA3-1183R	TCGGATCCTCCTTGGTCTGA TCACTGGCCTTTCGAGTCAC	1175	MP and CP
RNA1_298_ RACE	GCTTTCGAACACCTCTACGCACACA	—	Replicase protein 1a
RNA2_112_ RACE	TCGGGAGCCAAAGACTCACCGGCAT	—	RdRp
RNA3_568_ RACE	ACATGGCGTTGGATGGTCGGTCTGG	—	CP

primer with the PrimeSTAR (TAKARA Bio USA, Inc) kit as per the manufacturer's recommendations. One water sample was used as a negative control for PCR. Most of the bands observed were in the 400–1000 bp range, but amplification of longer (1–2 kb) products was also observed for some plant samples as well as for the negative control.

To increase the likelihood of virus discovery, aliquots of all amplified PCR samples were pooled (~90 µL) into one composite sample and cleaned up using AMPure beads (Beckman Coulter). In order to satisfy the cluster spacing and density parameters on a MiSeq flow cell (Illumina 2016), a two-step protocol was used with the 1:0.8 and 1:2.7 DNA:bead v/v ratios employed to remove <200 bp (adapter dimers, primer dimers and partial construct) and > 700 bp products, respectively. The 700 bp insert size is the extreme threshold of an acceptable range for the MiSeq flow cell and was chosen to enable the maximum recovery of sequences. Fragments were analysed in a 5 K DNA LabChip® GX Touch HT (PerkinElmer), resulting in the 230–700 bp size range; the concentration was 60.7 ng/

µL, as estimated by Broad Range Qubit fluorometric quantification (Thermo Fisher Scientific). A single adapter Nr 16 (5'-CCGTCCCG -3') from the Set A (KAPA Single-Indexed Adapter Kit, (KAPABIOSYSTEMS) was ligated to the composite sample using the KAPA HyperPrep kit (KAPABIOSYSTEMS) as per manufacturer's recommendations and the composite sample was sequenced as part of another library (0.0015x) on an Illumina MiSeq 250 bp single-end run at the Auckland Genomics Facility at the University of Auckland, New Zealand.

Sequencing data were analysed using a custom Claident-based pipeline (<https://www.claident.org/>). Binary base call files were parsed through bcl2fastq (v2.20, Illumina) and generate a total of 12,000 fastq sequences devoted to the composite sample. The composite sample was identified from the fastq sequences with the 5'-CCGTCCCG -3' index using the clsplitseq script from Claident (v0.2.2018.05.29). A total of 1615 sequences matched the index tag and were quality filtered, adapter trimmed, and exported to Geneious Pro version 10.8 (Kearse et al. 2012). De novo assembly was performed from the sequences using the built-in Geneious assembler with the High-Sensitivity Medium runtime method (variant merge coverage <6, no trimming, merge homopolymer variants, maximum gap per read 15%, minimum overlap identity 80%, minimum overlap 25 bp, word length 14, reanalyse threshold 8, maximum ambiguity 4, maximum mismatches per read 30%). A total of 1040 (64.3%) sequences assembled into 175 contigs with an average length of 447 ± 311 bp and 6 ± 12 reads per contig. The longest contig was 796 bp and consisted of 107 reads. The blastn analysis of contigs resulted in 107 plant, 22 bacterial, 16 fungal, 16 vertebrate, 9 bacteriophage and one algal sequences. The remaining four contigs (330–884 bp in length) were blastn (<http://www.ncbi.nlm.nih.gov>) searched against the all virus database (megablast algorithm, Organism limit: *viruses*, taxid: 10239), resulting in 78.1–88.8% nucleotide sequence identity AGLV-1998 nucleotide sequences JX463340.1, JX463341.1, and

Table 2 Ageratum latent virus New Zealand isolate (AGLV-NZ) amino acid (aa) and nucleotide (nt) sequence identity of with other known isolates

AGLV-NZ	Replication protein 1a	RdRp 2a	MP	CP
Length (aa)	914	594	294	237
Length (nt)	2742	1782	882	711
Completeness (%)	83.3	75.2	100	100
Amino acid sequence identity (%) with:				
AGLV-1998	86.8	85.9	89.1	87.4
AGLV-837	NA	NA	NA	84.2
Nucleotide sequence identity (%) with:				
AGLV-1998	82.4	82.2	81.6	83.7
AGLV-837	NA	NA	NA	82.1

JX463342.1. The blastn analysis of the unassembled 575 reads resulted in no hits to known viruses and it is possible that virus sequences with very low identity to known viruses were missed.

For analysis of the plant host of the virus, cDNA was generated using fresh total RNA extractions from all four plant hosts and amplified using the KAPA3G plant kit (Custom Science) with three pairs of diagnostic primers (Table 1), designed based on the four contigs using the Primer3 primer design feature of Geneious software (Rozen and Skaletsky 2000). The *V. bollonsii* sample was identified as a source of the virus; *B. f. compacta*, *M. bullata* and *L. scoparium* tested negative for the virus. Three PCR products were sequenced on an ABI Avant 3100 Genetic analyzer using BigDye 3.2 chemistry. After translation, the top 97.5%, 83%, 77.7% and 80.8% amino acid matches were to the AGLV-1998 (corresponding to the replicase protein 1a (JX463340.1), RdRp (JX463341.1), MP and CP (JX463342.1) respectively), confirming the virus origin.

For detailed characterisation of the virus sequence from *V. bollonsii* sample, 5'-end sequence recovery was performed using i) the SMARTer® RACE 5'/3' Kit (Takara Bio USA, Inc) with specific primers for the 5'-RACE (Table 1) and ii) generic Ilarvirus primers Ilar 1R7, Ilar 2R9 (Untiveros et al. 2010) and TSV RNA3-1F/TSV RNA3 1982 primers (Sharman and Thomas 2013). The 3'-end sequence recovery was unsuccessful. The total lengths obtained were 2761 nt for the RNA1 (MT474112), 1817 nt for the RNA 2 (MT474113) and 1953 nt for the RNA3 (MT474114) (76.7%, 63.3% and 87.8% completeness, respectively, compared to the full genome sequences).

AGLV was further identified in three individual samples: *V. dieffenbachia* (MT474115, 99.7% identity), *V. brevifacemosa* (MT474116, 99.2% nt identity) and *V. bollonsii* (MT474117, 99.7% nt identity). Total RNA extraction, reverse transcription, and PCR with the RNA1-39F/RNA1-408R primers was performed as above. Compared to the MT474112 sequence, all three virus amplicons had one 1-nt substitution resulting in the amino acid change (T vs S); there were two synonymous mutations (1-nt substitutions) in the amplicon from the *V. brevifacemosa*.

The new isolate of AGLV-NZ is described in this study for the first time in New Zealand and the first time on *V. bollonsii*, *V. brevifacemosa* and *V. dieffenbachia* based on the amino acid similarity of four genes (or their partial sequences) to the AGLV-1998 (85.9–89.1%) (Table 2). Further research on i) the prevalence of AGLV in other plant genera and other *Veronica* species, as well as ii) modes of transmission is necessary to demonstrate the potential impacts of this virus in New Zealand.

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