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First record of *White clover cryptic virus-2* in New Zealand

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Abstract
White clover cryptic virus-2 is recorded for the first time in New Zealand in three commercial cultivars of white clover: Grasslands Huia, Grasslands Kopu II and Grasslands Sustain. An RT-PCR assay was used to amplify and sequence a ~590 bp fragment of the coat protein gene.

Keywords White clover · Partitivirus · Cryptic virus

Plant cryptic viruses are distributed among three *Partitiviridae* genera: *Alphapartitivirus*, *Betapartitivirus* and *Deltapartitivirus* (Nibert et al. 2014). Although betapartitiviruses infect plants their genomes are related to partitiviruses that infect fungi. Representatives of two of these genera have been reported from New Zealand (Veerakone et al. 2015). Radish yellow edge virus is present, probably as a mixture of Alpha- and Delta- partitiviruses (Li et al. 2016), *Ryegrass cryptic virus* (RGCV, *Deltapartitivirus*) was detected in one cultivar of ryegrass (Veerakone et al. 2015) and *White clover cryptic virus-1* (WCCV-1, *Alphapartitivirus*) occurs at varying incidences (0–48%) across New Zealand (Guy and Gerard 2016). To date there have been no records of Betapartitiviruses.

The association of partitiviruses with their hosts is quite cryptic. These viruses only reach low titres in their hosts, and under certain conditions, they appear to be commensals or mutualists with their host rather than pathogens. Alpha- and Beta- partitiviruses may coexist in the same cell and coinfection with endornaviruses has been reported (Lesker et al. 2013). Other partitiviruses may infect fungi associated with the same host plant leading to complicated dsRNA patterns and the need to exercise caution when assigning new partitiviruses to plant or fungal hosts (Lesker et al. 2013; Nibert et al. 2014). WCCV-1 may have a role in the regulation of the host-rhizobium symbiosis (Nakatsukasa-Akune et al. 2005). The expression of RGCV was upregulated in perennial ryegrass when plants were grown under saline conditions (Li et al. 2012) however it is uncertain whether this benefits or harms the host.

As a first step in studying Betapartitiviruses in New Zealand we concentrated on *White clover cryptic virus-2* (WCCV-2) which was likely to occur here because Boccardo et al. (1987) detected WCCV-2 in single or mixed infections with WCCV-1 in unnamed cultivars from various parts of the world.

Total RNA was extracted from leaves (10-20 mg) or seeds (5–20) of white clover cultivars using the Trizol (Sigma Chemicals) method was used in the Superscript IV™ One-Step RT-PCR system with Platinum SuperFi™ DNA polymerase (Invitrogen, Auckland).

WCCV-2 isolate IPP Lirepa, segment RNA 2, (Lesker et al. 2013) complete sequence (GenBank Accession JX971977.1) and the NCBI Primer-Blast program were used to design WCCV-2 specific primers (WCCV-2 RNA-2F: ATCGGCCC CCGAAAGAATTT and WCCV-2 RNA-2r: TCAAATCC AGCAGCAGAGGG). RT incubations were at 50 °C and the annealing temperature for PCR was 64 °C. WCCV-2 was readily detected in leaf and seed extracts. WCCV-2 was detected in three cultivars of white clover: all the Grasslands Huia (5/5) and the Grasslands Kopu II (7/7) but only one of the Grasslands Sustain (1/30) plants were infected. Grasslands Huia and Grasslands Kopu II parentage contains New Zealand ecotypes whereas Grasslands Sustain's parentage contains some New Zealand germplasm as well as Mediterranean and USA ecotypes (Caradus 1986). The interaction between germplasm and virus is worthy of further investigation.

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Two amplicons of the predicted size (~590 bp) were sequenced using the *ABI 3730xl DNA Analyser* system. Forward and reverse sequences were assembled and edited (final size 533 bp) using the Geneious 11.0.4 program (Biomatters, Auckland) and compared with NCBI Genbank using a BLASTn search. The Huia sequence (GenBank Accession MH796795) and the Kopu II sequence (GenBank Accession MH796796) showed 93% identity with each other and both showed 94% identity with the one WCCV-2 sequence (WCCV-2 isolate IPP Lirepa) on GenBank.

A total of three cryptic viruses have been detected in white clover. This study confirms that, in addition to the alphapartitivirus WCCV-1, the betapartitivirus WCCV-2 occurs in New Zealand. WCCV-3 has not been sequenced and there are no commercially available antisera, therefore, reliable detection and characterization of this virus awaits the application of next generation sequencing to the limited number of white clover lines believed to be infected with this virus (Boccardo et al. 1987).

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