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First report of *Turnip vein clearing virus* and *Ribgrass mosaic virus* from New Zealand

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Abstract Leaves of *Plantago* spp. growing in two regions of New Zealand were sampled and tested for the presence of tobamoviruses. Transmission, immunological and molecular studies revealed the presence of both *Turnip vein clearing virus* (TVCV) and *Ribgrass mosaic virus* (RMV). The phylogenetic analysis of complete coat protein amino acid sequences grouped these viruses in the RMV and TVCV clusters of sub group 3 tobamoviruses. This is the first confirmed report of these viruses from New Zealand.

Keywords Tobamovirus · Plantain · Ribgrass mosaic virus · Turnip vein clearing virus · New Zealand

Ribgrass mosaic virus (RMV), *Turnip vein clearing virus* (TVCV), *Youcai mosaic virus* (YoMV) and *Wasabi mottle virus* (WMoV), belong to subgroup 3 of the genus *Tobamovirus*. This subgroup have a common host range, serological cross-reactivity and amino acid composition of their coat proteins compared with tobamoviruses in subgroups 1 and 2 (Heinze et al. 2006). Although TVCV and RMV have been reported from most parts of the world, they have not previously been reported to be present in New Zealand (Pearson et al. 2006). Plantago species have a worldwide distribution and are a natural host of TVCV, RMV and YoMV. Since *Plantago lanceolata* and *P. major* are common introduced weeds in New Zealand, it was thought likely that if TVCV and/or RMV were present in NZ, they would be present in these plants. In 2009 and 2010 plants of *P. major* and *P. lanceolata* growing in an orchard in the Bay of Plenty (BOP) were surveyed for viral-like symptoms and a further survey was carried out in 2010 from a vineyard in Hawke's Bay (HB), New Zealand. Preliminary tests were conducted in the field on extracts of leaves showing mild chlorosis or mottles, using the tobacco mosaic virus (TMV) Immunostrip from Agdia (ISK 57400), which can detect many tobamoviruses including RMV. Positive results were obtained from some plants collected at both sites indicating the presence of a tobamovirus. The positive samples from BOP were all *P. major*. From the HB, positive samples were detected from *P. major* and one of the *P. lanceolata* plants gave a weak positive.

Leaves from 86 plants of *P. major* and *P. lanceolata* collected from the BOP site were tested in an antigen-coated-plate ELISA format with an in-house TMV antiserum that can also detect RMV (Chavan et al. 2009). Ten of 71 *P. major* plants (14 %) gave positive results, whereas none of the 15 *P. lanceolata* plants tested positive. Extracts from seven of the *P. major* leaves that tested positive by ELISA and three of the *P. major* leaves that had tested negative were ground in phosphate buffer (0.1 M phosphate buffer, pH 7.5 containing 5 % polyvinylpyrrolidone (Sigma PVP40T) and 10 mM sodium sulphite) and mechanically inoculated to *Nicotiana occidentalis*. All seven extracts from plants that had tested positive by ELISA induced local lesions followed by severe systemic collapse of the upper leaves. The plants began to show recovery after about 2 weeks; although chlorosis and some dark green islands were seen on leaves as they expanded. No symptoms were seen on plants inoculated with the ELISA negative samples.

In order to identify whether the tobamovirus-positive samples were infected with TVCV and/or RMV, RNA was extracted from 100 mg leaf samples of *P. major*, *N. occidentalis* ex *P. major* and the *P. lanceolata* sample from HB that gave a weak positive, using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich Corp., St Louis, MO, USA) kit.

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Reverse transcription and polymerase chain reaction were carried out using SuperScript™ One step RT-PCR System with Platinum^{R} *Taq* DNA polymerase (Cat. No.12574-026) according to the manufacturer's protocol. A 1026 bp product comprising partial movement protein gene, complete coat protein (CP) gene and partial 3′UTR was amplified, using primers RMV primers, RM 9 F forward primer (5′ GAAACCGTGGCAAGTTCAT3′) and RM 10R reverse primer (5′CGGGGTTAGGGAGGATTC3′) which correspond to nucleotides 5275–5296 and 6283–6301, respectively of RMV sequence GQ401366. The gel purified DNA products were cloned and sequenced. Forward and reverse sequences from each PCR product were edited (to remove primer sequences) and consensus sequences were created using Sequencher 4.5 (Gene Codes Corporation, Michigan 48108, USA).

Complete tobamovirus CP sequences were obtained from nine infected *P. major* and used for phylogenetic analysis (Fig. 1). Sequences were deposited in GenBank with identical sequences deposited under a common accession number (see legend of Fig. 1). A partial CP sequence (201 nucleotides) was also obtained from the HB *P. lanceolata* sample. NCBI BLAST analysis of complete CP sequences indicated that eight sequences from BOP and HB isolates, represented by accessions HQ389329, HQ389331, HQ389332, HQ389333, and HQ389334, showed 92–97 % nt and 96–98 % aa identity to TVCV isolates. One sequence (accession HQ389330) from BOP was most similar to RMV isolates with 93–97 % nt and 96–99 % aa identity. The partial CP sequence from *P. lanceolata* was identical to HQ389331 and was therefore identified as TVCV.

Phylogenetic analysis (MEGA4, Tamura et al. 2007) of complete CP amino acid sequences from the New Zealand isolates from *Plantago sp.* with 17 representative CP sequences from sub group 3 and six representative sequences from sub groups 1 and 2 tobamoviruses showed that all of the New Zealand sequences clustered in the crucifer infecting sub group 3 tobamoviruses (Fig. 1). Sub group 3 tobamoviruses can be subdivided into RMV, TVCV and YoMV clusters based on phylogenetic analysis of the CP open reading frame (Heinze et al. 2006). Eight of the New Zealand isolates were placed in the TVCV cluster and one in the RMV cluster of sub group 3, confirming the above results. TVCV and RMV are closely related viral species and, in addition to *Plantago spp*., infect a range of cruciferous crops as well as *Impatiens*, *Digitalis*, *Lilium*, *Penstemon*, *Actinidia* and *Dichondra*, (Cardin et al. 2009; Chavan et al. 2009; Heinze et al. 2006; Lockhart et al. 2008). This is

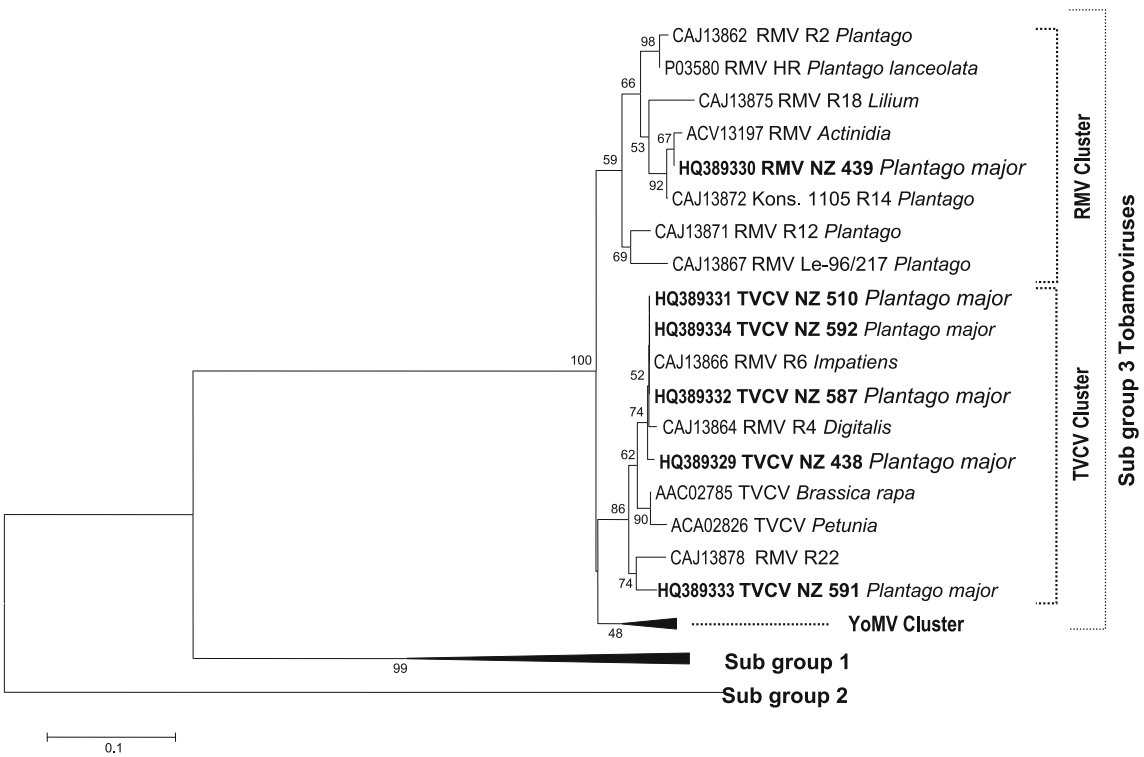

Fig. 1 Phylogenetic relationship of complete coat protein amino acid sequences of New Zealand TVCVand RMV isolates (*in bold*) with representatives of the three sub groups of genus *Tobamovirus* (Condensed NJ tree: tree topology derived using NJ method with Poisson corrected aa distances, pairwise gap deletion and uniform rate of evolution options. The nodal significance was evaluated based on 10000 Bootstrap random replicates. Tobamovirus sub group 1 and 2, and YoMV (*Youcai mosaic virus*) cluster of sub group 3 are condensed). N.B. GenBank accession HQ389332 represents isolates NZ 587 and NZ 588; HQ389334 represents NZ 592, NZ 593 and NZ 594

the first report of TVCV infecting *P. major* and *P. lanceolata* and RMV infecting *P. major* in New Zealand.

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References

Cardin L, Delecolle B, Moury B (2009) First report of *Cucumber mosaic virus* and *Turnip vein-clearing virus* in *Dichondra repens* in France, Italy and China. Plant Disease 93:201

Chavan RR, Pearson MN, Cohen D (2009) Partial characterization of a novel Tobamovirus infecting *Actinidia chinensis* and *A. deliciosa* (Actinidiaceae) from China. Eur J Plant Pathol 124:247–259

Heinze C, Lesemann D-E, Ilmberger N, Willingmann P, Adam G (2006) The phylogenetic structure of the cluster of tobamovirus species serologically related to *Ribgrass mosaic virus* (RMV) and the sequence of streptocarpus flower break virus (SFBV). Arch Virol 151:763–774

Lockhart BE, Swenson AS, Olszewski NE, Voth P (2008) Characterization of a strain of Turnip vein-clearing virus causing red ringspot of Penstemon. Plant Disease 92(5):725–729

Pearson MN, Clover GRG, Guy PL, Fletcher JD, Beever RE (2006) A review of the plant virus, viroid and mollicute records for New Zealand. Australas Plant Pathol 35:217–252

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetic analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599