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New host records and new host family range for *Turnip mosaic virus* in New Zealand

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Abstract. *Turnip mosaic virus* (TuMV) was reported in New Zealand for the first time in 1936. Until recently, TuMV had been found in nine hosts in this country. Using transmission electron microscopy, mechanical inoculation of herbaceous indicators, and molecular and serological techniques as detection and identification methods, another five new plant host records are reported from plants collected during surveillance and in post-entry quarantine. The new plant hosts infected with TuMV in New Zealand are *Crocus sativus* (Saffron), *Erodium moschatum* (Musky storkbill), *Lobelia speciosa* (Lobelia), *Nasturtium officinale* (Watercress) and *Tropaelum majus* (Nasturtium). The sequence analysis of the amplicons of TuMV indicates that the virus exists in a population of variants in New Zealand.

Turnip mosaic virus (TuMV, family *Potyviridae*, genus *Potyvirus*) was first identified in New Zealand in 1936 (Chamberlain 1936). Until recently nine plant species had been reported as hosts of TuMV in this country: *Armoracia rusticana*, *Brassica napus*, *B. pekinensis*, *B. rapa*, *Erysimum cheiri*, *Lunaria annua*, *Matthiola incana*, *Sinapis alba* and *Sisymbrium officinale* (Pearson *et al*. 2006). Five new plant host records are reported here from plants collected during surveillance and in post-entry quarantine during 2002–2005. The plants in post-entry quarantine were subsequently cleared for entry into New Zealand.

Samples were examined for the presence of virus by transmission electron microscopy (TEM) using negatively stained crude sap preparation as previously reported (Milne 1993). Herbaceous indicators (*Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Gomphrena globosa*, *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa*, *N. occidentalis* and *N. tabacum* cv. White Burley) were mechanically inoculated with sample crude sap as described by Lebas *et al*. (2005). A TuMV-specific double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) from Agdia Inc. (Elkhart, USA) was used to test the plants for the presence of the virus. Double-stranded RNA analysis (dsRNA) was performed using the method proposed by Valverde *et al*. (1990) and Lane (2003).

To further characterise TuMV, reverse transcription–polymerase chain reaction (RT-PCR) using *Potyvirus* broad detection primer pairs (Langeveld *et al*. 1991; Pappu *et al*. 1993) targeting the *Potyvirus* 3′-end region was carried out. Total RNA was extracted from leaves of the original hosts and symptomatic herbaceous-indicator plants using the Qiagen RNeasy® plant mini kit (Qiagen Inc., Chatworth, USA). RT was performed using random hexamer primers and SuperScript II RNase H^- reverse transcriptase (Invitrogen, Frederick, USA). Subsequently, the PCR products were either directly sequenced or cloned using TOPO TA cloning kit (Invitrogen), then sequenced, followed by NCBI-BLASTn alignment. A diagnostic segment of 242 bp was used for phylogenetic analysis.

TuMV was found infecting *Crocus sativus* (Saffron) from Mid-Canterbury (South Island), *Erodium moschatum* (Musky storkbill) from Auckland (North Island), *Lobelia speciosa* (Lobelia) from Auckland but imported from the UK, *Nasturtium officinale* (Watercress) from Kumeu (North Island), and *Tropaelum majus* (Nasturtium) from Auckland. The symptoms observed on *C. sativus*, *E. moschatum*, and *N. officinale* single infected with TuMV varied from chlorosis, mild to severe mosaic, and necrosis (Fig. 1*a*, *b*, *d*). TEM results showed the presence of *Potyvirus*-like particles in both original hosts and symptomatic mechanically inoculated herbaceous indicator plants. Local lesions on *C. amaranticolor* and systemic symptoms with necrosis on *C. quinoa* were observed from each mechanical inoculation (data not shown). Local lesions and systemic necrosis were induced in all inoculated *Nicotiana* species. *C. sativus* and *G. globosa* did not express symptoms (data not shown). All herbaceous plant symptoms are consistent with TuMV infection (Provvidenti 1983). However, two specimens were found co-infected with multiple viruses. Sap from *L. speciosa* induced systemic necrosis in *C. sativus* and systemic chlorosis in *C. amaranticolor* and *C. quinoa*. This specimen was found to be co-infected with *Arabis mosaic virus* (ArMV, *Nepovirus*) and *Cucumber mosaic virus* (CMV, *Cucumovirus*) (Fig. 1*c*). All results from mechanical inoculation agreed with the TuMV, ArMV and CMV descriptions (Provvidenti 1983; Murant 1985; Rybicki 1995). ArMV and CMV identifications were further confirmed by DAS-ELISA. Similarly, *T. majus* plants expressing severe mosaic symptoms (Fig. 1*e*) were found to be co-infected with *Broad bean wilt virus 1* (BBWV-1), *Verbena latent virus* (VeLV) and an unidentified *Carmovirus* (Tang *et al*. 2006).

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Fig. 1. (*a*) Chlorosis observed on *Crocus sativus* infected with *Turnip mosaic virus* alone. (*b*) Mosaic, chlorosis and necrotic spots observed on *Erodium moschatum* infected with *Turnip mosaic virus* alone. (*c*) Mottling observed on *Lobelia speciosa* infected with *Arabis mosaic virus*, *Cucumber mosaic virus* and *Turnip mosaic virus*. (*d*) Severe mosaic observed on *Nasturtium officinale* infected with *Turnip mosaic virus* alone. (*e*) Severe mosaic observed on *Tropaeolum majus* infected with *Broad bean wilt virus 1*, *Turnip mosaic virus*, *Verbena latent virus* and an unidentified *Carmovirus*.

N. officinale was previously reported as a new host of TuMV in Venezuela (Marys and Carballo 2002); having found TuMV infecting the same host in New Zealand confirms the report of this new host-virus association. Double stranded RNA (dsRNA) patterns obtained from original hosts and symptomatic herbaceous indicator plants were consistent with the dsRNA pattern previously observed for potyviruses in our laboratory (data not shown). TuMV infection was, moreover, confirmed by DAS-ELISA from original hosts and symptomatic herbaceous indicator plants.

The 242 bp TuMV diagnostic sequences (partial capsid protein) obtained from *Crocus sativus*, *E. moschatum*, *L. speciosa*, *N. officinale* and *T. majus* show 96–99% nucleotide identity with TuMV isolates belonging to the world-B group which is the most geographically dispersed group containing TuMV isolated mostly from *Brassica* spp. (Tomimura *et al.* 2004). *Crocus sativus* and *E. moschatum* TuMV isolates are closest to TuMV isolate CHN12 from China (unknown host, NCBI accession AY090660) whereas *N. officinale* TuMV isolate is most similar to *Raphanus sativus* TuMV isolate NDJ from Japan (NCBI accession AB093616) and to *Brassica napus* TuMV isolate UK1 from the United Kingdom (NCBI accession AF169561) while *T. majus* TuMV isolate is most similar to *Lactuca sativa* TuMV isolate DEU7 from Germany (NCBI accession AB188980) (Fig. 2).

The total number of TuMV host records in New Zealand from present and previous reports is fourteen. The new host records belong to the families Brassicaceae, Campanulaceae,

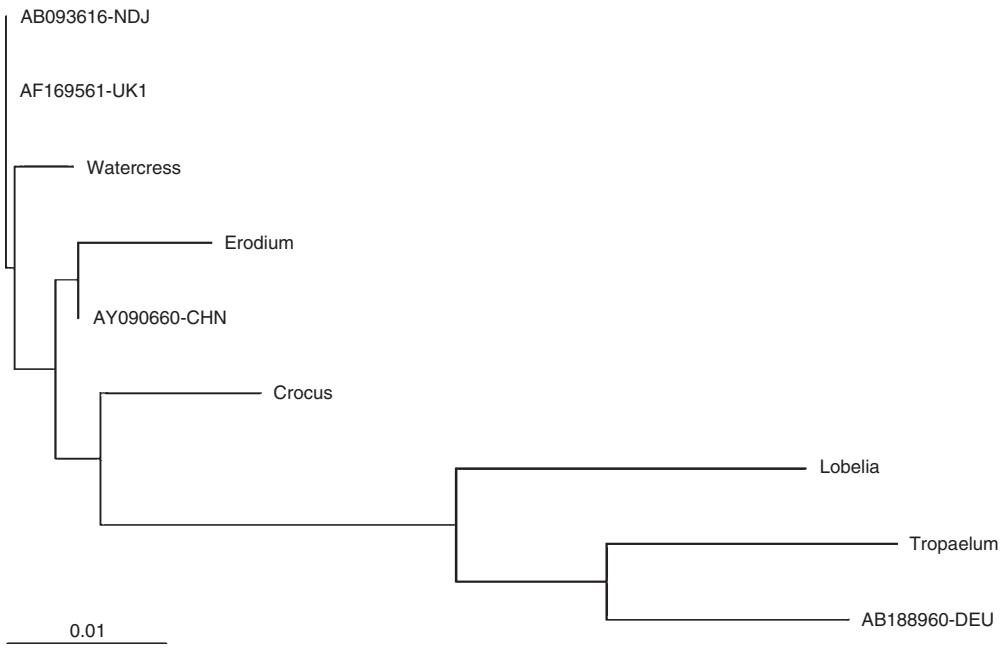

Fig. 2. Phylogeny of a 242 bp capsid protein diagnostic amplicon from TuMV infected hosts in New Zealand that shows the diversity of TuMV isolates found in different hosts.

Geranieaceae and Iridaceae. Because the previously reported nine plant hosts infected with TuMV were in the family Brassicae, the host records in the Campanulaceae, Geraniaceae and Iridaceae represent an extended range of plant families that are hosts of TuMV in New Zealand.

It was reported that seven TuMV isolates collected from *Brassica* spp. in New Zealand aligned within the world-B group (Ohshima *et al*. 2002). The partial 3′-terminus region sequence of the TuMV amplicons obtained in this study aligned with TuMV isolates detected from hosts other than *Brassica* spp., which also belongs to the world-B group (Tomimura *et al*. 2004). Interestingly, *N. officinale* TuMV isolate is similar to *Raphanus sativus* TuMV isolate NDJ from Japan (NCBI accession AB093616) which is a world-B/Asian-BR recombinant type with the proteinase, nuclear inclusion b (NIb) and coat protein genes belonging to the world-B group while the rest of the genome is part of the Asian-BR group (Tomimura *et al*. 2003). Similarly, *T. majus* TuMV isolate is closest to *Lactuca sativa* TuMV isolate DEU7 from Germany (NCBI accession AB188980), a world-B/basal-B recombinant type with recombination in the protein 1 gene only (Tomimura *et al*. 2004).

TuMV is not transmitted by seed but is transmitted in a non-persistent manner by 40–50 insect species. *Myzus persicae* and *Brevicoryne brassicae* (Aphididae) are the most efficient vectors (Provvidenti 1983), both of which are present in New Zealand (Cottier 1953). The ability of TuMV to be vectored by numerous aphid species facilitates the movement of this virus to new hosts. TuMV can cause serious biological damage to its hosts such as *Brassica* spp. (Tomlinson 1970) and on *Crocus sativus* (Fig. 1*a*), *Erodium moschatum* (Fig. 1*b*), and *Nasturtium officinale* (Fig. 1*d*) as reported here. TuMV has the ability to form recombinants which are widespread and can lead to emerging TuMV isolates with new biological properties (Ohshima *et al*. 2002; Tomimura *et al*. 2004). An increase in the number of hosts infected by TuMV in New Zealand is expected.

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