First report of *Narcissus mosaic virus* from Australia and from *Iris*

Stephen J. Wylie · Hua Li · Jiani Liu ·
Michael G. K. Jones

Received: 27 November 2013 / Accepted: 3 April 2014 / Published online: 18 April 2014
© Australasian Plant Pathology Society Inc. 2014

Abstract A plant of *Iris xiphium* in Western Australia displayed chlorotic streaks on the leaves and spathe that resembled symptoms of virus infection. After sequencing total RNA extracted from the plant, a sequence of 6,957 nt representing the complete genome of an Australian isolate of *Narcissus mosaic virus* (NMV) was obtained. It shared 97 % nucleotide identity with the two available complete genome sequences of NMV. This is the first report of natural infection of *Iris* by NMV.

Keywords Flower bulb · Virus incursion · Next generation sequencing · Biosecurity · Vegetative propagation · Virus accumulation

Narcissus mosaic virus (NMV) is a member of the genus *Potexvirus* (family Alphaflexiviridae). It was first described in Britain from *Narcissus* (family Amaryllidaceae) in 1966 (Brunt 1966). In 1989, the type isolate of NMV from The Netherlands was one of the first plant virus genomes to be fully sequenced (GenBank accession D13747) (Zuidema et al. 1989). Later, it was described from New Zealand (Clark and Guy 2000). In 2001 a partial genome sequence (AJ270988) from the Netherlands was released, and in 2003 another complete genome from New Zealand became available (AY225449). Most records of NMV are from narcissus, but it is recorded to naturally infect *Nerine* and *Hippeastrum*, both of which are also members of the family Amaryllidaceae

(Koenig et al. 1973), *Crocus* (Iridaceae) (Miglino et al. 2005), *Lilium* (Liliaceae) (Bellardi et al. 1988), and it is recorded as transmissible to a number of experimental host plant species including *Nicotiana benthamiana* and *N. clevelandii* (Solanaceae), *Chenopodium amaranticolor*, *C. quinoa*, and *Gomphrena globosa* (Amaranthaceae) (Brunt et al. 1996; Zuidema et al. 1989).

Here we report the detection and sequencing of the complete genome of an isolate of NMV infecting *Iris xiphium* Desf. cv Telstar Blue (Spanish Iris, Dutch Iris) (Iridaceae) in Australia. An iris bulb was purchased in 2013 from a retail hardware store in Perth, Western Australia, and grown within an insect-free greenhouse. The leaves and spathe displayed mild streaking patterns resembling those of some virus infections. Leaf material from this plant was macerated in cold 0.1 M phosphate buffer (pH 7.0) and mixed with diatomaceous earth before manually applying the mixture to five seedling plants each of *Nicotiana benthamiana* (accession RA-4), *N. umbratica* (accession We-1), *Chenopodium amaranticolor* and *Gomphrena globosa*. The same number of plants were mock-inoculated with buffer and diatomaceous earth. Chlorotic local lesions appeared on inoculated leaves of five *C. amaranticolor* plants within 7 days of inoculation. On *G. globosa*, faint pink spots appeared on inoculated leaves of 3/5 plants inoculated after 12 days. Symptoms of systemic infection in five plants each of *N. benthamiana* and *N. umbratica* were mild mosaic and slight leaf distortion on uninoculated emerging leaves 12–21 days after inoculation. Mock-inoculated plants did not display these symptoms. Interestingly, Zhang et al. (Zhang et al. 2013) reported that *N. benthamiana* plants displayed very mild symptoms of systemic infection with a strain of NMV from New Zealand (12), whereas the Australian strain described here appears to induce somewhat stronger symptoms on this host.

Previously we used a high-throughput sequencing approach to identify isolates of *Ornithogalum mosaic virus*, *Iris*

S. J. Wylie (✉) · H. Li · M. G. K. Jones
Plant Virology, Western Australian State Agricultural Biotechnology
Centre, School of Veterinary and Life Sciences, Murdoch University,
Perth, WA 6150, Australia
e-mail: s.wylie@murdoch.edu.au

J. Liu
Integrated Pest Management, Agricultural Faculty of Kunming
University, Kunming, Yunnan Province, China

mild mosaic virus, and *Japanese iris necrotic ring virus* infecting *Iris* species in Australia (Wylie et al. 2012a, b), and in this case a similar procedure was followed. Briefly, total RNA was extracted from a symptomatic leaf of the same plant used to inoculate experimental host plants (above), and cDNA was synthesized using random primers before library preparation. High-throughput sequencing was done on a Roche GS Junior machine. Adaptor sequences were removed and sequence reads were edited where necessary before contigs were assembled de novo using CLC Genomics workbench software v6.0.1. Blastn and Blastx analysis revealed a sequence of 6,957 nucleotides (nt) that shared 97 % nt identity across its complete genome with those of the Dutch (6,955 nt) and New Zealand (6,956 nt) isolates of NMV, confirming its identity as an isolate of *Narcissus mosaic virus*. Mean length of sequence reads corresponding to the NMV genome was 451 nt, with a range of 200–801 nt (reads of less than 200 nt were eliminated from the analysis). Mean sequence coverage over the virus genome was 17-fold.

The new isolate was designated NMV-SW13-Iris. Like other NMV isolates, the NMV-SW13-Iris genome is predicted to encode a replicase with a mass calculated to be 184.8 kDa, a triple gene block involved in viral movement consisting of three proteins of 25.9 kDa, 12.8 kDa, and 11.1 kDa translated from overlapping reading frames, a coat protein (CP) of 24.4 kDa, and a protein of unknown function of 10.1 kDa expressed from within the CP gene in the +1 reading frame. The nucleotide sequence of the NMV-SW13-Iris genome was granted Genbank accession KF752593. Only one RNA virus, NMV, was detected in the symptomatic plant tested.

This virus was previously described infecting members of at least five monocotyledonous plant genera representing three families. Although NMV has been identified in *Crocus*, a genus in the family Iridaceae, the significance of this discovery is that NMV also naturally infects at least one species within its sister genus *Iris*. The presence of NMV in Australia has not previously been reported. This finding has implications for the production of healthy iris plants by the floriculture and horticulture industries, in addition to potential biosecurity implications for the international trade in iris bulbs and flowers. A range of viruses infects *Iris*, a genus that is generally clonally propagated through bulbs and tubers.

Vegetatively propagated plants are susceptible to an accumulation of viruses over (vegetative) generations, and some species, such as garlic (*Allium sativum*), *Hippeastrum* and *Narcissus* species may be simultaneously infected with numerous viruses (Wylie et al. 2010, 2011; Wylie and Jones 2012). Thus, it will not be surprising if future studies of other species of *Iris* reveal the presence of NMV.

References

- Bellardi MG, Marani F, Bertaccini A (1988) Narcissus mosaic virus in lily. In VII International Symposium on Virus Diseases of Ornamental Plants 234:457–464
- Brunt AA (1966) Narcissus mosaic virus. Ann Appl Biol 58:13–23
- Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L (1996) Viruses of plants. descriptions and lists from the VIDE database. CAB International, Wallingford
- Clark VR, Guy PL (2000) Five viruses in *Narcissus* spp. from New Zealand. Australas Plant Pathol 29:227–229
- Koenig R, Lesemann D, Brunt AA, Kühne H (1973) Narcissus mosaic virus found in *Nerine bowdenii*. Identification aided by anomalies in SDS PAGE. Interviol 1:348–353
- Miglino R, Jodłowska A, Van Schadewijk AR (2005) First report of narcissus mosaic virus infecting *Crocus* spp. cultivars in the Netherlands. Plant Dis 89:342
- Wylie SJ, Jones MGK (2012) Complete genome sequences of seven carlavirus and potyvirus isolates on *Narcissus* and *Hippeastrum* plants in Australia, and proposals to clarify their naming. Arch Virol 157:1471–1480
- Wylie SJ, Nouri S, Coutts BA, Jones MGK (2010) Narcissus late season yellows virus and *Vallota speciosa* virus found infecting domestic and wild populations of *Narcissus* species in Australia. Arch Virol 155:1171–1174
- Wylie SJ, Li H, Jones MGK (2011) Phylogenetic analysis of allexiviruses identified on garlic from Australia. Australas Plant Dis Note. doi:10.1007/s13314-011-0038-2
- Wylie SJ, Li H, Jones MGK (2012a) First report of an isolate of Japanese iris necrotic ring virus from Australia. Australas Plant Dis Note. doi:10.1007/s13314-012-0059-5
- Wylie SJ, Luo H, Li H, Jones MGK (2012b) Multiple polyadenylated RNA viruses detected in pooled cultivated and wild plant samples. Arch Virol 157:271–284
- Zhang H, Wang L, Hunter D, Voogd C, Joyce N, Davies K (2013) A Narcissus mosaic viral vector system for protein expression and flavonoid production. Plant Meth 9:28
- Zuidema D, Linthorst HJ, Huisman MJ, Asjes CJ, Bol JF (1989) Nucleotide sequence of narcissus mosaic virus RNA. J Gen Virol 70:267–276