

Tobacco mild green mosaic virus in *Impatiens* and *Osteospermum*: new hosts and first report in the UK

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In May 2007 two samples of *impatiens* (*Impatiens* sp.) (cv. Timor and Totoya) exhibiting possible virus symptoms were received from a nursery in the south of England. Symptoms included stunting, distorted leaves, necrotic lesions and a paler flower colour. ELISA tests for *Cucumber mosaic virus*, *Impatiens necrotic spot virus* and *Tomato spotted wilt virus* were negative. Mechanical inoculation onto indicator plants produced symptoms on *Chenopodium quinoa* (local chlorotic lesions), *Nicotiana benthamiana*, *N. hesperis* and *N. occidentalis* P1 (all systemic necrosis). Examination of these indicator plants and the original samples by electron microscopy revealed straight rod-shaped particles (approximately 300 nm in length). This strongly suggested the presence of a tobamovirus. Therefore, the samples were tested using a tobamovirus PCR (Agdia) and bands of the correct size (400 bp) were detected. The products were cloned and sequenced (GenBank Accession No. GU77405) and the virus identified as *Tobacco mild green mosaic virus* (TMGMV).

Following this finding of TMGMV in *impatiens*, a sample of *osteospermum* (*Osteospermum* sp.) (cv. Sheila) was received in March 2008 from a second nursery in the south of England. The sample had leaf symptoms including chlorotic spots and rings. Testing by ELISA was negative for several common viruses including *Cucumber mosaic virus*, *Impatiens necrotic spot virus*, *Lettuce mosaic virus* and *Tomato spotted wilt virus*. The same virus symptoms as observed for the *impatiens* samples were detected on indicator plants following mechanical inoculation; examination by transmission electron microscopy revealed suspected tobamovirus particles. PCR was done using the same tobamovirus PCR (Agdia); following sequencing the virus was identified as TMGMV (GU74404).

TMGMV was first reported in *N. glauca* from the Canary Islands (McKinney, 1929). There have been a few reports of TMGMV in *Capsicum* in Asia and Central/South America, the first being in Korea (Choi *et al.*, 2002). It has also been reported in Israel on *Torenia fournieri* (Scrophulariaceae), *Calibrachoa* spp. and *Petunia* spp. (Solanaceae) (Zeidan *et al.*, 2008). Other natural hosts include *N. tabacum* cv. Samsun, *Eryngium aquaticum* and *E. planum* (Brunt *et al.*, 1996). This is the first report of TMGMV in the UK and also the first reported findings in the families Balsaminaceae and Asteraceae. The symptoms of TMGMV on both *impatiens* and *osteospermum* make infected plants unsaleable. There is also a concern that infected ornamental plants may act as a reservoir for infection of *Capsicum*.

References

- Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ, 1996. *Plant Viruses Online: Descriptions and Lists from the VIDE Database*. Wallingford, Oxfordshire, UK: CABI Publishing.
- Choi GS, Kim JH, Ryu KH *et al.*, 2002. First report of *Tobacco mild green mosaic virus* infecting pepper in Korea. *The Plant Pathology Journal* 18, 323–7.
- McKinney HH, 1929. Mosaic diseases in the Canary Islands, West Africa and Gibraltar. *Journal of Agricultural Research* 39, 557–78.
- Zeidan M, Beckelman E, Holdengreber P *et al.* 2008. Detection of *Tobacco mild green mosaic virus* in ornamental crops. In: *Proceedings of the 12th International Symposium on Virus Diseases of Ornamental Plants, Haarlem, the Netherlands, 20–24 April 2008*. 52.

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Identification of *Mirafiori lettuce big-vein virus* and *Lettuce big-vein associated virus* infecting *Lactuca sativa* with symptoms of lettuce big-vein disease in Argentina

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Lettuce big-vein disease (BVD) affects all major lettuce-producing areas of the world. The causal agent is *Mirafiori lettuce big-vein virus* (MLBVV), an ophiiovirus transmitted by the soil-borne fungus *Olpidium brassicae* (Lot *et al.*, 2002). MLBVV has been detected in many different areas of the world but never in Argentina. La Plata has about 700 ha of lettuce with a production of about 13 000 tonnes, and with about 70% of the total production from Buenos Aires Province. BVD has been detected in different areas in the north and west of the La Plata horticultural green belt. Many of the plants with BVD symptoms had leaf distortions of moderate severity, which affected their commercial value. Over the winters of 2007, 2008 and 2009, forty samples of lettuce with BVD symptoms, representing most of the commercial varieties, were taken from fields near La Plata. Samples tested positive by RT-PCR with universal primers for the genus *Ophiiovirus* (Vaira *et al.*, 2003) and/or by DAS-ELISA using a polyclonal antiserum against the viral coat protein of MLBVV (kindly provided by Dr Y. Kawazu, National Institute of Vegetable and Tea Science, Japan).

The identity of MLBVV was verified by cloning and sequencing amplicons (GenBank Accession Nos. FJ555204-05), which shared 97% identity with both Italian (AY204674-1) and Brazilian (DQ854813-1) MLBVV isolates. Specific primers, Cps1 (5'-CTCATGACAAAAGAA

GAGAAGC) and Cpas1 (5'-CACATCAAATATGAAGTTGTGCTC) were designed from MLBVV-RNA 3, to allow specific MLBVV detection in RNA extracts from collected samples. The primers were tested using samples with symptoms that were positive by RT-PCR and DAS-ELISA. An amplicon of the expected 450 bp was amplified from all infected samples. Sequence analysis (GU295451) demonstrated high identity (98%) with sequences deposited in the public databases. A block sampling method was employed to collect random field samples and estimate the incidence of the disease. These were scored for symptoms and tested for infection by RT-PCR (primers Cps1/Cpas1). In the three lots tested, the incidence of the disease reached 60% of plants inoculated. Additionally, soil transmission experiments were conducted using healthy lettuce seedlings planted into contaminated soil or by watering with rinse water from the roots of diseased field plants. After 5 weeks in a greenhouse up to 70% of the 15 seedlings inoculated with five field isolates showed big-vein symptoms and tested positive by DAS-ELISA and RT-PCR. Three of the plants used for transmission were also positive for *Lettuce big-vein associated virus* (LBVaV), detected by RT-PCR using specific primers (VP248 and VP249; Navarro *et al.*, 2004). This is the first report confirming the presence of MLBVV and LBVaV infecting lettuce plants in Argentina.

Acknowledgements

The authors would like to thank Dr. L.W. Timmer for helpful discussion and manuscript revision.

References

Lot H, Campbell RN, Souche S, Milne RG, Roggero P, 2002. Transmission by *Olpidium brassicae* of *Mirafiori lettuce virus* and *Lettuce big-vein virus*, and their roles in lettuce big-vein etiology. *Phytopathology* 92, 288–93.

Navarro JA, Botella F, Maruhenda A, Sastre P, Sanchez-Pina MA, Pallas V, 2004. Comparative infection progress analysis of *Lettuce big-vein virus* and *Mirafiori lettuce virus* in lettuce crops by developed molecular diagnosis techniques. *Phytopathology* 94, 470–7.

Vaira AM, Accotto GP, Costantini A, Milne RG, 2003. The partial sequence of RNA 1 of the ophiovirus *Ramunculus white mottle virus* indicates its relationship to rhabdoviruses and provides candidate primers for an ophiovirus-specific RT-PCR test. *Archive of Virology* 148, 1037–50.

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Plant Pathology (2010) 59, 1161

Doi: 10.1111/j.1365-3059.2010.02294.x

First report of a new subgroup 16SrIX-E ('*Candidatus* Phytoplasma phoenicium'-related) phytoplasma associated with juniper witches' broom disease in Oregon, USA

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Western juniper (*Juniperus occidentalis*) is a native tree indigenous to parts of Oregon, Washington, Idaho, Nevada and California (USA). The tree has increased in density since settlement of these areas, raising concern over loss of understory plants, decreased wildlife habitat and increased soil erosion. A newly recognized disease, juniper witches' broom (JunWB), affecting at least 1% of trees in central Oregon, is characterized by abnormal proliferation of shoots, reduced size of leaves, shortened internodes, and growths having a ball-like appearance. DNA was extracted from leaf samples from ball-like growths and used as template in polymerase chain reactions primed by primer pair P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995). DNA fragments of 1.8 kb amplified from two samples were sequenced and the sequences deposited in GenBank (Accession Nos. GQ925918 and GQ925919). RFLP patterns of 16S rDNA, observed as virtual patterns using *iPhyClassifier* (Zhao *et al.*, 2009), indicated that JunWB phytoplasma represents a new subgroup lineage, designated 16SrIX-E. 16S rDNA sequence similarity confirmed that JunWB is a '*Ca. Phytoplasma phoenicium*'-related phytoplasma.

JunWB is one of three phytoplasmas found thus far to infect gymnosperms and is the only phytoplasma known to infect *Juniperus* sp. Occurrence of three distinct phytoplasmas, JunWB phytoplasma (this study), a group 16SrIII strain, and '*Ca. Phytoplasma pini*' (Schneider *et al.*, 2005), in gymnosperms of two different families (Pinaceae and Cupressaceae,

Division Coniferae) in Europe and North America, suggests that phytoplasma infection of conifers may be more common than previously envisioned.

References

Deng S, Hiruki C, 1991. Amplification of 16S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* 14, 53–61.

Schneider B, Seemüller E, Smart CD, Kirkpatrick BC, 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S, Tully JG, eds. *Molecular and Diagnostic Procedures in Mycoplasma*. New York, NY, USA: Academic Press, Vol. 2, 369–80.

Schneider B, Torres E, Martin MP, Schröder M, Behnke H-D, Seemüller E, 2005. '*Candidatus* Phytoplasma pini', a novel taxon from *Pinus silvestris* and *Pinus halepensis*. *International Journal of Systematic and Evolutionary Microbiology* 55, 303–7.

Zhao Y, Wei W, Lee I-M, Shao J, Suo X, Davis RE, 2009. Construction of an interactive online phytoplasma classification tool, *iPhyClassifier*, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *International Journal of Systematic and Evolutionary Microbiology* 59, 2582–93.

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