



## First report of *Broad bean wilt virus 2* in *Veronica gentianoides* in the UK

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In August 2016 a sample of *Veronica gentianoides* cv. Tissington White was submitted to Fera Science Ltd. from a nursery in West Sussex. The sample was submitted with symptoms of severe distortion and mottling and these symptoms were widespread throughout the nursery which could affect their marketability. The sample was tested by ELISA for *Cucumber mosaic virus* (Agdia, USA), *Arabidopsis mosaic virus*, potyviruses, *Tobacco ringspot virus*, *Tomato ringspot virus* and *Tomato spotted wilt virus* (DSMZ, Germany), *Impatiens necrotic spot virus* and *Strawberry latent ringspot virus* (Bioreba, Switzerland). The sample was negative for all viruses tested. The sample was subsequently tested for *Tobacco rattle virus* using RT-PCR with specific primers (Mumford *et al.*, 2000) but also tested negative.

To check for the presence of virus the sample was then mechanically inoculated onto *Chenopodium quinoa*, *Nicotiana benthamiana*, *N. hesperis* and *N. occidentalis* P1. Thirteen days post inoculation all plants showed symptoms such as chlorotic spots, distortion and necrosis (Figs. 1-2).

RNA was extracted from symptom-bearing leaves of *N. benthamiana* using an RNeasy Plant Mini Kit (Qiagen, UK), and a sequencing library prepared using the Scriptseq complete plant root kit (Illumina, UK) and sequenced along with other indexed samples on an Illumina MiSeq using a 2x300 bp V3 kit. 61,857 paired reads were produced and analysed as described by Adams *et al.* (2014). Ten contigs totalling 8,236 bp were identified having between 94-96% identity at the nucleotide level to substantial parts of the bipartite genome of *Broad bean wilt virus 2* (BBWV-2). The protein sequence of the combined coat proteins had between 88-98% identity to that of coat proteins of different isolates of BBWV-2 found on GenBank. Figure 3 shows a neighbour joining tree (1000 replicates) produced using MEGA6 (Tamura *et al.*, 2013) of the coat protein genes of this isolate (GenBank Accession No. MF429951) compared to other members of the family *Secoviridae*. This confirms it as a member of the species BBWV-2.

To confirm the identification, material from *N. benthamiana* was tested by ELISA (DSMZ, Germany; reference number AS-0862, and LOEWE, Germany; reference number 07017S) using a positive control (PC-0862)

and was found to be positive with both antisera.

BBWV has previously been found on *V. scutellata* in New York (Rist & Lorber, 1989) though it was not specified whether BBWV-1 or BBWV-2 was present. BBWV-2 has been previously detected in *Digitalis* and *Salvia officinalis* in the UK (Mumford *et al.*, 2006). This is thought to be the first confirmed finding of BBWV-2 in *Veronica*.

### Acknowledgements

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Figure 1



Figure 2

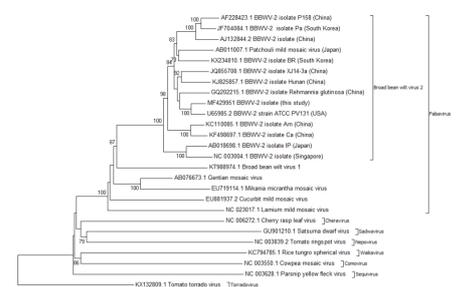


Figure 3

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