



First report of Rose spring dwarf-associated virus in *Rosa* spp. in United Kingdom

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In July 2019, a sample of *Rosa* spp. was submitted to Fera Science Ltd. via the Royal Horticultural Society (RHS) gardening advice service. The sample (ID220) was sent in following the appearance of unknown symptoms including mottling, yellow/white patching, thin texture and a pink colour in the leaves.

RNA was extracted using a CTAB method adapted from Adams *et al.* (2009), with the 4M LiCl incubation performed overnight at 4°C. The sample was tested for common rose viruses using RT-qPCR (Table 1). A positive result was achieved for Rose cryptic virus 1. Subsequently, the sample was analysed by high throughput sequencing (HTS) using a TruSeq Stranded Total RNA Library Prep Plant kit (Illumina Inc., USA) for library preparation. A MiSeq instrument and a MiSeq Reagent Kit v3 (600-cycle) (Illumina Inc.) were used to run the library. The run generated 569,452 reads for the sample, and data was analysed as described by Fox *et al.* (2019). Three fragments of rose spring dwarf-associated virus (RSDaV) were identified (234, 251 and 229 bp; GenBank Accession Nos. MT993839-MT993841). A BLAST+ search found sequences with high sequence identity in both nucleotide (92.11-93.59% identity, EU024678.1) and amino acid comparisons (94.34-100%, YP_001949737.1; YP_001949736.1; YP_001949738.1). RT-PCR amplification using specific primers (Salem *et al.*, 2008) was performed to confirm the result, and a product of the expected size (418 bp) was obtained.

To assess the spread of RSDaV in the UK, 171 roses were analysed using the RT-PCR assay. Samples were collected as part of a survey of rose viruses in the UK and both asymptomatic and symptomatic leaf samples, consistent with virus infections symptoms (mottling, yellow veining, distortion, and ringspots) were included. Only one sample (ID140) resulted positive for RSDaV, and no symptoms were identified. Previous analysis showed this sample was positive for *Arabis mosaic virus* by ELISA and RT-qPCR.

The RT-PCR product (418 bp) from both RSDaV-positive samples (ID220, 140) were sequenced, and nucleotide comparisons showed a 98.51-99.02% identity with sequences in GenBank (HM236366.1; HM236364.1; HM236362.1; HM236364.1). Amino acid comparison showed a 98.51-100% identity with previously published sequences (FAM78852.1; ADK78851.1).

RSDaV has previously been found in the USA (Salem *et al.*, 2008), Chile

(Rivera & Engel, 2010), and New Zealand (Milleza *et al.*, 2013). This is the first report of RSDaV in Europe. Further samples (4) were submitted to the RHS and Fera Science Ltd. Plant Clinic, showing the previously described unknown symptoms. They were tested by RT-PCR and also sequenced by HTS and tested negative for RSDaV. The cause of these symptoms is not believed to be of viral origin.

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References

- Adams IP, Glover RH, Monger WA, Mumford R, Jackeviciene E, Navalinskiene M, Samuitiene M, Boonham N, 2009. Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. *Molecular Plant Pathology* **10**, 537-545. <http://dx.doi.org/10.1111/j.1364-3703.2009.00545.x>
- Fox A, Fowkes AR, Skelton A, Harju V, Buxton-Kirk A, Kelly M, Forde SMD, Pufal, H, Conyers C, Ward R, Weekes R, Boonham N, Adams IP, 2019. Using high-throughput sequencing in support of a plant health outbreak reveals novel viruses in *Ullucus tuberosus* (Basellaceae). *Plant Pathology* **68**, 576-587. <http://dx.doi.org/10.1111/ppa.12962>
- Milleza EJM, Ward LI, Delmiglio C, Tang JZ, Veerakone S, Perez-Egusquiza Z, 2013. A survey of viruses infecting *Rosa* spp. in New Zealand. *Australasian Plant Pathology* **42**, 313-320. <http://dx.doi.org/10.1007/s13313-012-0191-x>
- Rivera PA, Engel EA, 2010. Presence of rose spring dwarf-associated virus in Chile: partial genome sequence and detection in roses and their colonizing aphids. *Virus Genes* **41**, 295-297. <http://dx.doi.org/10.1007/s11262-010-0510-7>
- Salem N, Golino DA, Falk BW, Rowhani A, 2008. Identification and partial characterization of a new luteovirus associated with rose spring dwarf disease. *Plant Disease* **92**, 508-512. <http://dx.doi.org/10.1094/pdis-92-4-0508>

Table 1. Specific primers used in this study

Virus	Genus	Primers (F/R)	Reference
Apple mosaic virus	Betaviruses	AppleV1-F: TCC TCC AAG ATT AAG AAT GAA GTA AppleV1-R: TGA ACC ATT TCG GCG CAG	Mohamed <i>et al.</i> (2017). <i>Plant Dis</i> 101 :18877. doi:10.1094/pldis-2017-101-18877
Arabis mosaic virus	Xenoviruses	AMV-F: TGG CCG TCG GAG GCA ATC TC AMV-R: CAG CAG AAT TCG ACA TTA TC	Wu <i>et al.</i> (2013). <i>Australasian Plant Pathology</i> 42 , 59-66. doi:10.1007/s13313-011-0095-7
Cucumber mosaic virus	Cucumoviruses	CMV-F: GCT TCG TCG GCG TCG A CMV-R: AAG GCA GCA ACT TTA GCA ACT GT CMV-R2: TGA AGG TAT TCG AAC TCG AAG C	Shahin <i>et al.</i> (2018). <i>New Disease Reports</i> 9 , 25. doi:10.5197/j.2044-0588.2018.09.025
Popillia necrotic spot virus	Zeacoviruses	PNV-F: CAG TCG TCG CCA CAA ACC TCG AAG C PNV-R: TTA TCG ATT CTT GAG GAA GAA PNV-R2: TCG TCG CAG AAC AAG CAG CAG C	Development for Entomoculture, Food & Rural Affairs (2005) Integrated management of viruses and fungi vectors in protected horticulture. UK. 2005. 12-020.
Prunus necrotic ring-spur virus	Betaviruses	PNRV-F: AAG TCG GCG TCG CCG TCG TCG TCG PNRV-R: CAG TCG GCG TCG CCG TCG TCG TCG	Marlet <i>et al.</i> (2010). <i>Plant Disease</i> 94 , 1344-1346. doi:10.1094/pdis-94.13.1344
Raspberry ring-spur virus	Xenoviruses	RRSV-F: TCG TCG TCG TCG TCG TCG TCG RRSV-R: TCG TCG TCG TCG TCG TCG TCG	Mague & Maudsloni (2010). http://horticulture.ahis.org.uk/sites/default/files/research_pages/SP_39
Rose necrotic virus I (girdling agent)	Alphaviruses	RNV-F: TCG TCG TCG TCG TCG TCG TCG RNV-R: TCG TCG TCG TCG TCG TCG TCG	Vannote-Gilbert <i>et al.</i> (2010). <i>Journal of Plant Pathology</i> 90 , 1171-1173. doi:10.1007/s11262-010-0607-5
Rose necrotic virus II	Enamoviruses	RNV2-F: CAG TCG TCG TCG TCG TCG TCG RNV2-R: TCG TCG TCG TCG TCG TCG TCG	This study
Shepherdia necrotic ring-spur virus (Sheep Shearers)	Changraviruses	SSRV-F: CAG TCG TCG TCG TCG TCG TCG SSRV-R: TCG TCG TCG TCG TCG TCG TCG	Mague & Maudsloni (2010). http://horticulture.ahis.org.uk/sites/default/files/research_pages/SP_34
Tahoe necrotic virus	Zelkoviruses	TNV-F: TCG TCG TCG TCG TCG TCG TCG TNV-R: TCG TCG TCG TCG TCG TCG TCG	Maudsloni <i>et al.</i> (2005). <i>Phytopathology</i> 95 , 448-451. doi:10.1094/pdis-2005-95-448
Tahoe ring-spur virus	Xenoviruses	TRSV-F: CAG TCG TCG TCG TCG TCG TCG TRSV-R: TCG TCG TCG TCG TCG TCG TCG	EPPO (2017). <i>Bulletin of European Plant Pathology</i> , 47 , 133-140. doi:10.1111/epp.12274
Tahoe ring-spur virus II	Xenoviruses	TRSV2-F: CAG TCG TCG TCG TCG TCG TCG TRSV2-R: TCG TCG TCG TCG TCG TCG TCG	Tang <i>et al.</i> (2014). <i>Journal of Plant Pathology</i> 33 , 18-43. doi:10.1007/s13313-014-0232-2
Texas groundhull virus	Zeacoviruses	TGV-F: CAG TCG TCG TCG TCG TCG TCG TGV-R: TCG TCG TCG TCG TCG TCG TCG	EPPO (2006). <i>Bulletin of European Plant Pathology</i> 34 , 273-278. doi:10.1111/j.1365-3113.2006.00702.x

Figure 1

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