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RESEARCH NOTE

First report of iris yellow spot virus infecting shallot (*Allium cepa* var. *aggregatum*) in Australia

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Abstract

In 2019, symptoms typical of infection by iris yellow spot virus (IYSV; family *Tospoviridae*, genus *Orthotospovirus*) were observed on shallot (*A. cepa* var. *aggregatum*) grown at the Gatton Research Facility, Department of Agriculture and Fisheries, Queensland. Initial testing by DAS-ELISA was positive for IYSV, and subsequent specific RT-PCR amplifying part of the RdRp (L-segment) and sequencing of amplicons confirmed the IYSV infection. This is the first record of IYSV infection of shallot in Australia.

Keywords ELISA · IYSV · Orthotospovirus · RdRp · Tospoviridae

Iris yellow spot virus (IYSV; *Orthotospovirus iridimaculaflavi*, family *Tospoviridae*) is an economically important pathogen of allium crops (Resende and Pappu 2021). IYSV was first described and characterised in 1998 in the Netherlands from Dutch iris (*Iris* × *hollandica*) with chlorotic symptoms. (Cortês et al. 1998). However, the disease had previously been recognised in onion (*Allium cepa* var. *cepa*) but misdiagnosed. An orthotospovirus that was erroneously identified as tomato spotted wilt virus was associated with diamond shaped lesions on the scapes of onion in Brazil in 1981, and the disease called "sapeca" (de Ávila et al. 1981). It was later shown that this virus was serologically distinct from known orthotospoviruses (Pozzer et al. 1994). In the summer of 1989, similar symptoms were found on onions in Treasure Valley, USA, and the causal agent was subsequently identified as IYSV (Moyer et al. 2003).

IYSV has an ambisense, tri-partite, single-stranded RNA genome, with segments designated as large (L), medium (M), and small (S) based on their length (Resende and Pappu 2021). Onion thrips (*Thrips tabaci*) and tobacco thrips (*Frankliniella fusca*) transmit the virus in a circulative propagative manner (Kritzman et al. 2001; Rotenberg et al. 2015; Srinivasan et al. 2012). The virus is acquired by first and second larval instars, transmitted through the feeding by adult thrips, and localised lesions appear at the feeding site (Birithia et al. 2013). In onion the infection by IYSV becomes systemic (Kritzman et al. 2001) while in leek it remains localised (Smith et al. 2006). IYSV is most commonly detected in alliums, including onion, leek (*A. ampeloprasum*), chives (*A. schoenoprasum*), shallot (*A. cepa* var. *aggregatum*), garlic (*A. sativum*), and green onion (*A. fistulosum*).

IYSV causes distinct symptoms in onion that are commonly described as "straw bleaching" with diamond shaped lesions and necrotic eye-like spots on the infected leaf or flower scape (Watson et al. 2011). Slightly different symptoms, described as stroke-shaped lesions on the leaf, have been reported from shallot (Huchette et al. 2008). The host range of IYSV extends to monocot and eudicot ornamental plants including Dutch iris (*Iris* × *hollandica*), *Hippeastrum hybridum* and lisianthus (*Eustoma russellianum*), and weeds, including redroot pigweed (*Amaranthus retroflexus*), smooth pigweed (*A. hybridus*), spiny pigweed (*A. spinosus*), bacon weed (*Chenopodium album*), jimsonweed (*Datura stramonium*), kochia grass (*Kochia scoparia*), and goose grass (*Eleusine indica*) (Ahsan and Ashfaq 2018; Bag et

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al. 2015; Karavina and Gubba 2016; Kritzman et al. 2000; Sampangi et al. 2007).

In Australia, IYSV was first recorded in 2002 from onions and leeks in the States of Western Australia, Victoria, and New South Wales (Coutts et al. 2003). In August 2019, we observed symptoms consistent with IYSV infection in onions in a planting of shallot cv. 'Tuk Tuk' at the Gatton Research Facility, Queensland Department of Agriculture and Fisheries (QDAF) (27°32'S; 152°22'E). Leaf and flower scape with diamond shaped lesions or necrotic spots were collected from separate shallot plants (Fig. 1A) and, following testing, were lodged in the QDAF Plant Virus Collection as isolates 5739 and 5740. In this study, we used verified IYSV reference isolate 3447 from onion grown at Gatton (Gambley 2022) as a positive control.

Initial diagnostic testing was conducted using an IYSV-specific DAS-ELISA (Agdia Inc., US, Cat No. SRA 60500/0500), following the manufacturer's protocol. Absorbance values were measured using a Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer at 405 nm (Thermo Fisher Scientific, Waltham, US). Confirmatory diagnostic testing was conducted by RT-PCR assay. Samples were ground and extracted using a Tissue Lyser and BioSprint 15 DNA Plant Kit (QIAGEN, Hilden, Germany) respectively, following the manufacturer's protocol except that the RNase enzyme was omitted from buffer RLT. Total nucleic acid extractions were tested using degenerate tospovirus primers (Andrew Geering, *unpublished*), TospoRp-F1 (5′-ATGGGIATITTYGAYTTYATGIGRYATGC-3′) and TospoRp-R1 (5′-GGCATCATRTAIATIGCIARRTAIACATT-3′), that amplify part of the RNA-dependent

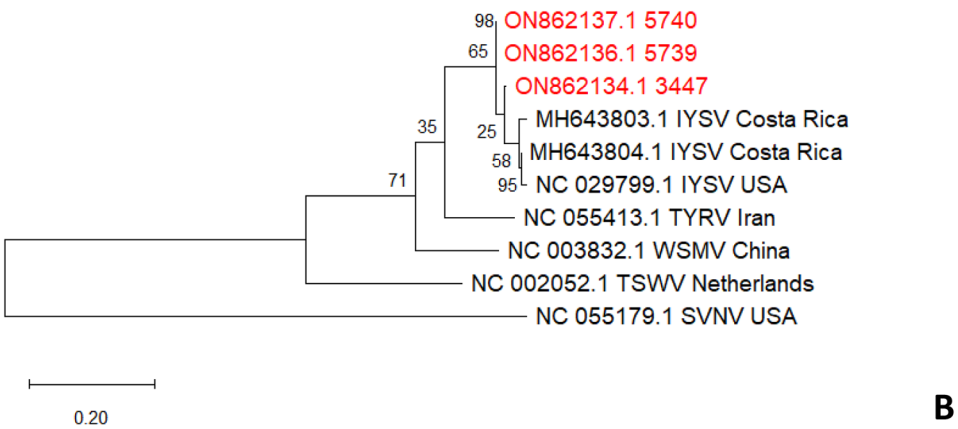

Fig. 1 **A**, Leaf and flower scape symptoms on fresh shallot (*Allium cepa* var. *aggregatum*) infected with isolates 5739 and 5740, respectively; **B**, Maximum-Likelihood phylogenetic tree of partial RdRp sequences of IYSV. Australian IYSV isolates are written in red and other orthotospovirus members were included for comparison. Abbreviations: SVNV, soybean vein necrosis virus; TSWV, tomato spotted wilt virus; TYRV, tomato yellow ring virus; WSMV, watermelon silver mottle virus

RNA polymerase (RdRp) gene on the L-segment. RT-PCR was conducted using the MyTaq™ One-Step RT-PCR Kit (Bioline Meridian Bioscience, Memphis, USA) as per the manufacturer's protocol. The reverse transcription step was conducted at 50 °C for 20 min, then reactions were incubated at 95 °C for 1 min to inactivate the reverse transcriptase and denature the RNA-DNA complex. This step was followed by 40 amplification cycles consisting of denaturation at 95 °C for 10 s, primer annealing at 52 °C for 10 s, extension at 72 °C for 30 s, and a final extension step of 72 °C for 2 min. Samples were electrophoretically separated through 1.5% agarose in 0.5 × TBE buffer and visualised following staining with ethidium bromide. Amplicons were gel extracted using the ISOLATE II PCR and Gel Kit (Bioline Meridian Bioscience, Memphis, USA) and then sent to Macrogen Inc., South Korea for Sanger sequencing. Bioinformatic analysis of sequences (including alignments) was conducted using Geneious Prime version 2020.2.4 (Biomatters Ltd., Auckland, New Zealand) and the BLAST suite of algorithms (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was generated using MEGA X (Kumar et al. 2018) and the Maximum-Likelihood algorithm was implemented with 1000 bootstrap replicates.

Isolates 5739 and 5740 were both positive for IYSV by DAS-ELISA and for orthotospoviruses by RT-PCR. The sequenced RdRp amplicons from isolates 5739 and 5740 were 100% identical across 262 nt. The amplicons from isolates 5739, 5740 and 3447 all had the same closest GenBank match to iris yellow spot virus (FJ623474.1), with 95.4%, 95.4% and 96.2% pairwise nucleotide identity, respectively. Phylogenetic analysis of Australian IYSV isolates with other currently available orthotospovirus L-segment sequences, based on the partial RdRp sequences, showed that the Australian isolates grouped together with IYSV isolates from USA and Costa Rica (Fig. 1B). The Australian IYSV sequences for isolates 5739, 5740 and 3447 were deposited in GenBank under accessions ON862134.1, ON862136.1 and ON862137.1, respectively.

IYSV isolates fall into two broad groups, Eurasia ($IYSV_{BR}$) and Americas ($IYSV_{NL}$), based on nucleocapsid and non-structural movement protein sequences (Bag et al. 2015; Silva et al. 2001). While the molecular and serological data for these two shallot orthotospovirus isolates shows they clearly belong to the species *Orthotospovirus iridimaculaflavi*, only the RdRp sequence is currently available for these isolates. Further sequence data from the nucleocapsid and movement protein genes would be needed to extend the comparison of these isolates with other Australian and international IYSV isolates.

In conclusion, this work extends the known host range of IYSV in Australia, providing the first record of the virus in shallot. To date, IYSV infecting shallot has only been reported in the USA (Pappu et al. 2006), New Zealand (Ward et al. 2009), and France (Huchette et al. 2008). The source of infection at Gatton is uncertain but is likely a non-allium host since only garlic, but not onion, leek or other allium crops were grown nearby, and garlic is very rarely recorded as a host of IYSV (Ahsan and Ashfaq 2018; Bag et al. 2009).

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Data availability The sequence data generated and analysed during the current study are available in the NCBI GenBank repository, https://www.ncbi.nlm.nih.gov/nucleotide/. Other datasets analysed in this study are available from the authors upon reasonable request.

Declarations

Ethical approval This article contains no studies with human participants or animals performed by any authors. Therefore, informed consent was not required.

Conflict of interest The authors declare that they have no conflict of interest.

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