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RESEARCH NOTE

Detection of two poleroviruses infecting garlic (*Allium sativum*) in Australia

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Abstract

Two near complete polerovirus genomes were assembled using high throughput sequencing (HTS) data from two separate samples of garlic cultivar 'Glenlarge' grown in Gatton, Queensland, Australia. Whole genome sequence comparisons showed that one contig shared 96.7% nucleotide identity with phasey bean mild yellows virus (MT966032.1) and the other, 99.8% nucleotide identity with turnip yellows virus (MT586581.1). Phylogenetic analyses further revealed that the isolates fell within the PBMYV group 2 and TuYV group 2 clades, respectively. This is the first report of these poleroviruses infecting garlic.

Keywords Garlic · High throughput sequencing · PBMYV · Polerovirus · TuYV

The genus *Polerovirus* is one of four genera in the family *Solemoviridae* and comprises viruses with monopartite, positive-sense, single-stranded RNA genomes of 5.6 – 6.2 kb, each containing seven open reading frames (ORFs) (Sõmera et al. 2021). The presence of ORF3a, ORF4, ORF6, and ORF7 distinguishes polerovirus genomes from those of related genera in the family *Solemoviridae* (Sõmera et al. 2021). Poleroviruses are phloem-limited (Peter et al. 2009) and commonly cause symptoms such as chlorotic streaking, leaf rolling and plant stunting (Garcia-Ruiz et al. 2020). In nature, poleroviruses are transmitted by one or more species of aphid in the persistent and circulative manner (Schliephake et al. 1999), although whitefly (*Bemisia tabaci*) transmission of a new polerovirus infecting bell peppers (*Capsicum annuum*) in Israel has recently been reported (Ghosh et al. 2019).

Nine officially recognized or tentative species of polerovirus are known from Australia, including phasey bean mild yellows virus (PBMYV) (Sharman et al. 2016) and *Turnip yellows virus* (TuYV) (Kehoe and Coutts 2019). Both viruses are broadly distributed across the country including Queensland (Sharman et al. 2021). PBMYV is primarily a pathogen of pulse legumes (Sharman et al. 2021), whereas TuYV has a much broader natural host range including brassica, legume, ornamental and native plant species (Geering and Thomas 2022; Jones et al. 2021). Earlier records of beet western yellows virus (BWYV) in Australia based on ELISA results are now considered erroneous due to the serological relatedness of this virus and TuYV: recent molecular analyses have revealed the presence of only TuYV in Australia (Filardo et al. 2021; Jones et al. 2021). Whole genome sequencing has shown that some isolates of PBMYV and TuYV have a recombinant ORF 5, although no biological differences due to the recombinations have been noted. Three distinct P5 groups of TuYV exist, of which group 2 is a recombinant between the other two sequence groups, labelled 1 and 3 (Filardo et al. 202). In contrast, only two P5 groups of PBMYV exist, and the donor virus giving rise to the recombinant ORF 5 in PBMYV group 2 (PBMYV-2) has not been identified (Sharman et al. 2021).

Garlic (*Allium sativum*) is a relatively minor crop in Australia, with approximately 3,360 tonnes produced domestically in 2020 and around 13,564 tonnes imported, mainly from China (AGIA 2021). Garlic in Australia is infected by many of the viruses that are common in this plant species worldwide, including potyviruses (onion yellow dwarf virus (OYDV) and leek yellow stripe virus (LYSV)), carlaviruses

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(garlic common latent virus (GCLV) and shallot latent virus (SLV)) and allexiviruses (garlic virus A – E, and X (GarVA – E, GarVX)) (Cremer et al. 2021; Nurulita et al. 2020; Wylie et al. 2014). These viruses were likely introduced to Australia in the initial germplasm imports and have perpetuated from one plant generation to the next as a result of vegetative propagation practices.

High throughput sequencing (HTS) technologies have revolutionized virus diagnosis and accelerated the rate of discovery of viruses that occur at a low titre in plant sap and are not easily detectable using traditional diagnostic techniques such as electron microscopy or inoculation onto biological indicator plants. We have applied HTS to investigate the diversity of viruses infecting garlic in Queensland and during the course of this study, detected PBMYV and TuYV for the first time in this plant species.

In 2019, leaf samples were collected from 60 'Glenlarge' garlic plants that were growing in a field trial at Gatton Research Station, the Department of Agriculture and Fisheries, Queensland, Australia. These leaf samples were sorted to create two batch samples, each containing single leaves from 30 plants, for analysis by HTS. All plants had chlorotic streaking on the leaves and some also had chlorotic lesions on the leaf sheaths. Total RNA was extracted using the TRIzol™ plus RNA Purification Kit (Invitrogen – ThermoFisher Scientific, Waltham, USA) and any minor DNA contamination removed by treatment with DNase I (New England Biolabs, Ipswich, USA) as per the manufacturers' protocols. Total RNA was sent to Australian Genome Research Facility (AGRF), Melbourne, Australia for library preparation, ribodepletion (TruSeq ribo depletion protocol with Ribo-Zero Plant kit, Illumina), and 100 bp paired-end high throughput sequencing (HTS) with an Illumina NOVASeq SP Lane 300 cycle kit. Following quality checking and trimming, de novo assembly was performed using the Galaxy web platform (https://usegalaxy.org.au/) following the pipeline from Bester et al. (2021). Sequence contigs were annotated using the BLAST algorithm to search the NCBI Virus RefSeq database (downloaded on 22 July 2020). Phylogenetic analyses were performed using IQ-TREE 2 (Minh et al. 2020) on the Galaxy web platform (https://usegalaxy.org.au/) and the trees constructed using the Maximum-Likelihood algorithm with 1000 ultrafast bootstrap replicates and best-fit model according to AIC (TIM2 + F + R3) (Hoang et al. 2017; Kalyaanamoorthy et al. 2017). The bootstrap value with cut off > 50 was applied using TreeGraph version 2.15.0–887 beta (Stöver and Müller 2010) and visualized with FigTree (version 1.4.4, http://tree.bio.ed.ac.uk/software/figtree/, last accessed on 25 April 2022). Pairwise similarity matrices were created by aligning sequences using the MUSCLE algorithm with selected polerovirus sequences from GenBank.

A PBMYV-like sequence contig, which was 5,801 nucleotides (nt) long, was assembled from 7,307 of the 62,287,060 trimmed reads obtained for batch sample 1. This sequence contig contained all seven ORFs as expected, suggesting that it represented a near-complete polerovirus genome. The ORF1 and ORF3 proteins shared 86.2 – 96.4% and 95.6 – 100% amino acid (aa) identity, respectively, with homologous proteins from other PBMYV isolates from Australia (Table 1). However, pairwise sequence comparisons using the P5 protein revealed only 57.5 – 59.5% aa identity with PBMYV group 1 (PBMYV-1) isolates but 95.8 – 97.6% similarity with PBMYV-2 isolates (Table 1). In a phylogenetic analysis, the near complete genome of PBMYV from garlic (DAF plant virus collection isolate 5571, GenBank LC701522.1) grouped with PBMYV-2 isolates from

Table 1 Percentage protein similarity between the assembled genome of PBMYV from garlic (isolate 5751, GenBank accession number LC701522.1) and other representative PBMYV Australian isolates based on ORF1 (P1 protein; 663 aa), ORF3 (CP; 199 aa), and ORF5 (P5 protein; 455 aa)

Virus isolate	GenBank accession	PBMYV variant	Host	ORF1	ORF3	ORF5
ESPCL15	KT963000.2	1	Clover (*Trifolium subterraneum*)	88.7	97.5	59.5
NSWCP15	KT962999.2	1	Chickpea (*Cicer arietinum*)	86.2	95.6	59.3
5511	MK955806.1	1	Chickpea (*C. arietinum*)	88.8	97.0	57.5
5580–1	MT966031.1	1	Phasey bean (*Macroptilium lathyroides*)	96.4	97.0	59.1
MK108	MT966033.1	1	Lentil (*Lens culinaris*)	88.7	97.5	59.5
MK215	MT966038.1	1	Lentil (*L. culinaris*)	88.6	97.0	59.5
QLDPB13	MK801779.2	2	Phasey bean (*M. lathyroides*)	95.2	100	96.5
5580–2	MT966032.1	2	Phasey bean (*M. lathyroides*)	96.3	99.0	97.6
MK114	MT966034.1	2	Clover (*T. subterraneum*)	88.7	97.5	96.1
MK137	MT966035.1	2	Clover (*T. subterraneum*)	88.7	97.0	96.1
MK212	MT966036.1	2	Pea (*Pisum sativum*)	88.4	97.5	96.1
MK214	MT966037.1	2	Lentil (*L. culinaris*)	88.9	97.5	96.1
2015pulse5-8	MT966039.1	2	Pea (*Pisum sativum*)	89.0	97.5	95.8

phasey bean (*Macroptilium lathyroides*) (MT966032.1 and MK801779.2), pea (*Pisum sativum*) (MT966036.1 and MT966039.1), clover (*Trifolium subterraneum*) (MT966034.1 and MT966035.1), and lentil (*Lens culinaris*) (MT966037.1) (Fig. 1A), and these sequences shared 93.5 – 96.7% nt identity across the full length of the aligned sequences.

A TuYV-like sequence contig, which was 5,063 nt long, was assembled from 775 of the 54,156,580 trimmed reads obtained for batch sample 2. This sequence contig was less complete than that obtained for PBMYV, as an estimated 303 nt at the 5′ end of ORF0 was missing, and ORF1, which overlaps ORF0 but in a different translational reading frame, was also truncated by 146 nt. However, all other ORFs that were expected to occur were identified. In pairwise sequence comparisons, the protein products of ORF1 (P1 protein, truncated), ORF3 (CP), and ORF5 (P5 protein) had 91.8. – 100%, 93.6 – 100%, and 97.5 – 100% aa identity with homologous proteins of group 2 isolates of TuYV (TuYV-2; Table 2). Pairwise comparisons of P5 protein sequences showed that the TuYV isolate from garlic shared 46.4 – 61.0% and 79.7 – 80.4% aa identity with

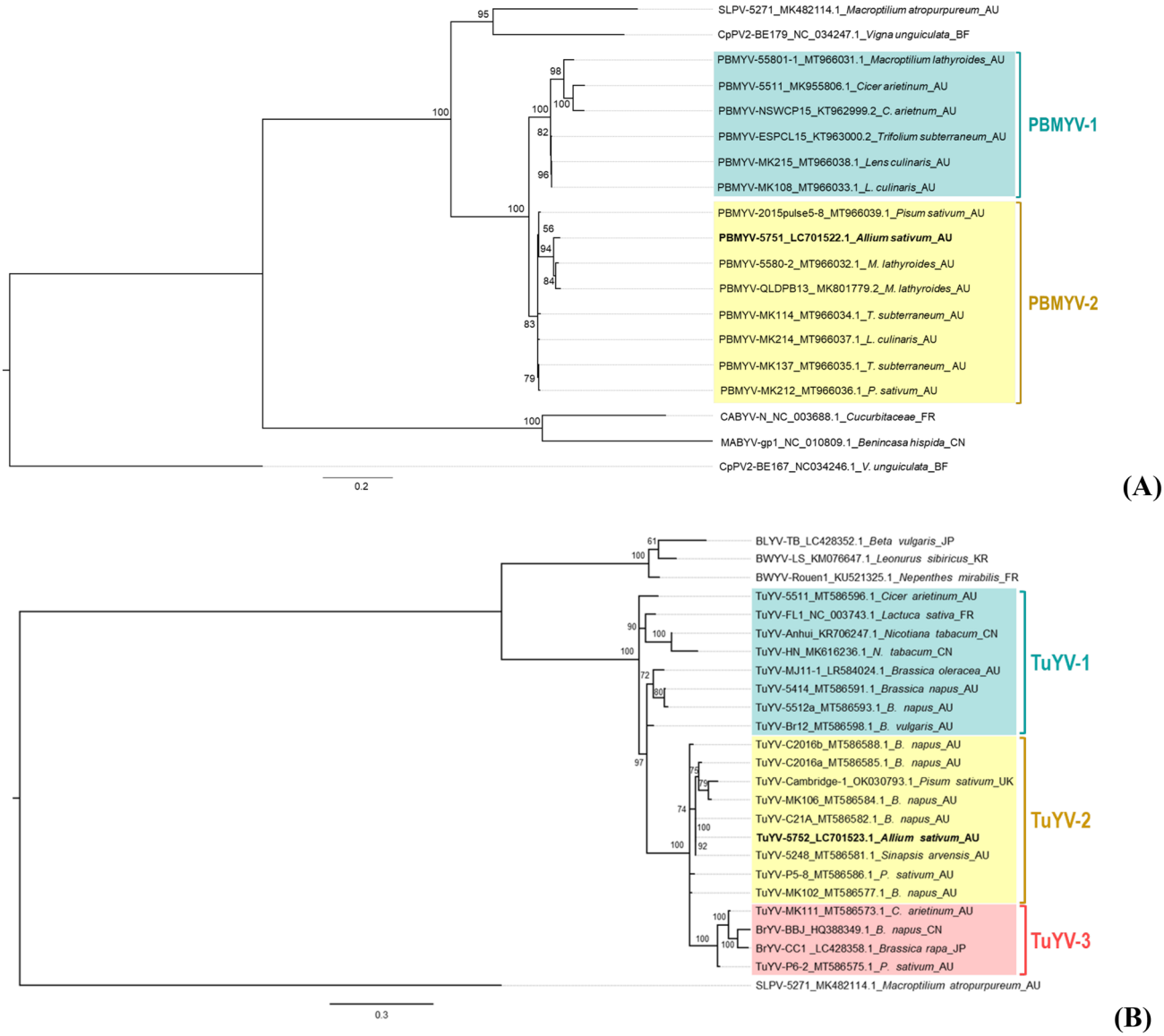

Fig. 1 Phylogenetic trees including PBMYV isolate 5751 (LC701522.1) (**A**) and TuYV isolate 5752 (LC701523.1) (**B**) from garlic with other representative isolates of these viruses. Trees were constructed from near complete genomes covering the 5642 nt and 5063 nt available from the garlic PBMYV and TuYV isolates, respectively, using the maximum-likelihood algorithm with 1000 replicates in IQ-TREE 2. The scale bar represents the number of nt substitutions per site. Garlic isolates are in bold, and siratro latent polerovirus (SLPV) is included as an outgroup

Table 2 Percentage protein similarity between the assembled genome of TuYV from garlic (isolate 5752, GenBank accession number LC701523.1) and other TuYV isolates based on partial ORF1 (P1 protein; 558 aa), ORF3 (CP; 202 aa), ORF5 (P5 protein; 434 aa)

Virus isolate	GenBank accession	TuYV variant	Host	ORF1	ORF3	ORF5
Anhui	KR706247.1	1	Tobacco (*Nicotiana tabacum*)	87.3	91.1	60.2
MJ11-1	LR584024.1	1	Broccoli (*Brassica oleracea*)	96.8	94.1	60.6
FL1	NC_003743.1	1	Lettuce (*Lactuca sativa*)	92.3	95.5	61.0
HN	MK616236.1	1	Tobacco (*N. tabacum*)	87.3	91.1	46.4
5414	MT586591.1	1	Canola (*Brassica napus*)	92.8	93.6	60.2
5512a	MT586593.1	1	Canola (*B. napus*)	90.5	94.1	60.4
5511	MT586596.1	1	Chickpea (*Cicer arietinum*)	89.1	92.6	58.9
Br12	MT586598.1	1	Beet (*Beta vulgaris*)	96.4	95.5	60.0
MK102	MT586577.1	2	Canola (*B. napus*)	95.9	97.5	99.3
5248	MT586581.1	2	Charlock (*Sinapsis arvensis*)	99.8	100	99.8
C21A	MT586582.1	2	Canola (*B. napus*)	100	100	99.5
MK106	MT586584.1	2	Canola (*B. napus*)	92.7	100	99.8
C2016a	MT586585.1	2	Canola (*B. napus*)	97.7	100	99.8
P5-8	MT586586.1	2	Pea (*Pisum sativum*)	96.4	100	100
C2016b	MT586588.1	2	Canola (*B. napus*)	95.7	100	99.8
Cambridge-1	OK030793.1	2	Pea (*P. sativum*)	91.8	93.6	97.5
BBJ	HQ388349.1	3	Canola (*B. napus*)	88.7	94.6	79.7
CC1	LC428358.1	3	Field mustard (*Brassica rapa*)	91.0	94.6	80.2
MK111	MT586573.1	3	Chickpea (*C. arietinum*)	96.6	94.6	80.4
P6-2	MT586575.1	3	Pea (*P. sativum*)	96.4	93.1	80.4

homologous proteins from TuYV-1 and TuYV-3, respectively. In a phylogenetic analysis, the TuYV isolate from garlic (DAF plant virus collection isolate 5572, GenBank LC701523.1) grouped together with TuYV-2 from charlock (*Sinapsis arvensis*) (MT586581.1), pea (*Pisum sativum*) (MT586586.1, OK030793.1), and canola (*Brassica napus*) (MT586577.1, MT586582.1, MT586584.1, MT586585.1, and MT586588.1) (Fig. 1B) and this sequence shared 94.1 – 99.8 nt identity across the aligned regions.

To determine the presence and incidence of infection of each of the poleroviruses, RT-PCR was done on leaf samples from individual plants from the original batches using a OneTaq® One-Step RT-PCR Kit (New England BioLabs, Ipswich, USA). Total nucleic acids were extracted using a BioSprint 15 Plant DNA kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions, except that RNase A was omitted from the extraction buffer. To detect PBMYV, the primers PBMYV2_5244F (5′-GACATGATAGAARCT TATCGAGAGTTA-3′) and PBMYV5828R (5′-CAACCC GGATTTCCATRTCC-3′) were used (Sharman et al. 2021) and for TuYV, the primers TuYV-2659F (5′-ATTACTTGA CGCCCACGGAT-3′) and TuYV-3052R (5′-GAGCAGAAT TCCAGTTGTCCG-3′) were used (Filardo et al. 2021). One out of 30 plants that constituted batch sample 1 was strongly positive for PBMYV, and likewise, one out of 30 plants that constituted batch sample 2 was positive for TuYV. Sanger sequencing of the PBMYV-2 specific PCR amplicon (579 nt) revealed 99.7% identity to the assembled genome, and for the TuYV-specific amplicon (300 nt), 99.6% identity to the assembled genome. Given the low incidence of infection of each of the viruses, it is most likely that the plants became infected within the current plant generation, and infection was not a result of vertical transmission through the cloves used to establish the crop.

PBMYV was first reported in Australia from chickpea (Liverpool Plains, NSW; KT962999.2), subterranean clover (Esperance, WA; KT963000.2), and phasey bean (Emerald, QLD; KT906372.1) (Sharman et al. 2016). More recently, a variant strain with a recombinant ORF 5 (PBMYV-2) was detected in Queensland, Australia from phasey bean (Mt McLaren, Kilcummin) and chickpea (Warwick and Jandowae regions) (Sharman et al. 2021). Garlic is the first recorded non-legume host for PBMYV-2 and only the second non-legume host record for PBMYV. Detection of TuYV-2 in garlic is the first instance of this P5 group in Queensland, although the two other P5 groups are recorded. Previously, TuYV-2 was only known from Victoria, New South Wales and Western Australia (Filardo et al. 2021).

These results expand the known host range of PBMYV and TuYV but do not represent the first records of a polerovirus infecting garlic as tobacco vein distorting virus was recently detected in garlic plants in Yunnan, China, also using a HTS approach (Tan et al. 2021). Further biological and field studies are needed to assess whether the viruses can be vegetatively transmitted in garlic or transmitted from garlic to other hosts by aphids, and whether they have an impact on

symptom expression or crop yield. A range of potyviruses (OYDV and LYSV), carlaviruses (SLV and GCLV) and allexiviruses (GarV A – E) were also detected in the bulked garlic plants we examined (data not shown), any of which could have contributed to the symptoms that were observed. There were no obvious differences in symptom expression in the samples containing the poleroviruses. To study symptomatology and yield impacts of the poleroviruses, it will be necessary to obtain virus-free garlic plants and do experimental inoculations.

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Declarations

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

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