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ORIGINAL RESEARCH ARTICLE

The identification of a novel cereal-infecting luteovirus, cereal red leaf virus, and clarification of other yellow dwarf viruses present in the Northern grain region of Australia

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Abstract

During a survey of oat and barley crops in northern New South Wales (NSW) and southern Queensland (Qld), a novel member of the genus *Luteovirus* (family *Tombusviridae*) causing leaf reddening and yellow dwarf symptoms in oats and barley was found in both States. A near-complete genome of the virus tentatively named cereal red leaf virus (CrRLV; tentative species name *Luteovirus foliarubra*) was obtained through high throughput sequencing. The virus was genomically distinct from, but reacted with antibodies to, barley yellow dwarf virus-MAV. CrRLV was transmitted by *Rhopalosiphon padi* but not by *R. maidis*. Barley yellow dwarf virus-PAV was found in both NSW and Qld on barley and oats. Additionally, barley virus G was found for the first time in Qld and NSW, on barley, barley yellow dwarf virus-PAS for the first time in Qld, on barley, cereal yellow dwarf virus-RPS for the first time in NSW, on oats, and cereal yellow dwarf virus-RPV was found in NSW on oats and barley and for the first time in Qld, on oats.

Keywords Luteovirus · Oats · Barley · Genome organization · Aphid vector · Polerovirus · Solemoviridae

Introduction

Cereal crops are important food sources worldwide. Yellow dwarf viruses (YDVs) infect many species in the family *Poaceae* and cause major yield reductions and economic losses in several crops, including wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rye (*Secale cereale*) and oats (*Avena sativa*) (Jones 2021; Miller and Lozier 2022; Nancarrow et al. 2021). These diseases are caused by at least 21 distinct phloem-limited, aphid-transmitted viruses belonging to the families *Tombusviridae* (genus *Luteovirus)* and *Solemoviridae* (genus *Polerovirus* and unclassified). Previously these viruses were grouped together in the former family *Luteoviridae*, which has now been abolished and its member species assigned to the families and genera above (Miller and Lozier 2022; Scheets et al. 2020). YDVs are characterised as having 25–30 nm diameter isometric virions (Miller & Rasochová, 1997), a positive sense single stranded RNA genome of *ca.* 5.7 kb and specific virus transmission by aphids in a circulative, non-propagative manner (Miller and Lozier 2022). YDVs in the *Tombusviridae* include barley yellow dwarf virus (BYDV)-PAV, -MAV, -OYV, -PAS, -kerII, -kerIII, -GAV and -SGV, and wheat luteovirus 1 while in the *Solemoviridae* these include barley virus G (BVG), cereal yellow dwarf virus (CYDV)-RPV, -RPS, maize yellow dwarf virus (MYDV)-RMV, wheat yellow dwarf virus (WYDV)-GPV, maize yellow mosaic virus, miscanthus

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Mark Schwinghamer is deceased. This paper is dedicated to his memory.

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yellow fleck virus, paspalum notatum polerovirus, rice dwarf polerovirus, panicum distortion mosaic virus, sugarcane yellow leaf virus, triticum yellow stripe virus and wheat leaf yellowing associated virus (ICTV 2023; Miller and Lozier 2022; Sõmera et al. 2021; Yan et al. 2024; Zhou et al. 2025).

Australia is a major grain-producing and exporting country, with production occurring in all States. Wheat is Australia's most valuable export crop ($16.7 billion) while barley ranks sixth ($3.3 billion) (Anonymous 2024). Australia is also ranked second to Cananda in oat exports (Anonymous 2023). YDVs cause significant yield losses in these crops and also affect a wide range of pasture grasses (Jones 2021). To date, BVG, BYDV-MAV, BYDV-PAS, BYDV-PAV, CYDV-RPV, CYDV-RPS and MYDV-RMV have been reported from Australia. Early identifications of YDVs in Australia were done predominantly using serological assays, sometimes supported by aphid vector transmissions. Using these methods, BYDV-MAV, BYDV-PAV, CYDV-RPV and MYDV-RMV were identified (Greber 1988; Guy et al. 1986, 1987; Johnstone 1995; McKirdy and Jones 1993; Sward & Lister 1988; Waterhouse and Helms 1985). More recently, BVG, BYDV-PAS and CYDV-RPS have been detected using PCR and sequencing (Nancarrow et al. 2019, 2023, 2024b). Two significant YDV vectors are not known to occur in Australia, viz. *Sitobion avenae* (wheat aphid) and *Schizaphis graminum* (spring green aphid) (Anonymous 2020, 2022). However, other aphid vectors including *Rhopalosiphum maidis*, *R. padi*, *Metapolophium dirhodum*, *Sitobion fragariae* and *Sitobion miscanthi* are present (Johnstone 1995).

Interestingly, Lister and Sward (1988) found YDV isolates in Victoria which were positive in BYDV-MAV-specific ELISA, but unexpectedly were readily transmitted by *R. padi*, not usually a recognised efficient vector of this YDV. In the present work, some samples of oats with typical yellow dwarf disease symptoms collected from northern New South Wales (NSW) and southern Queensland (Qld) gave positive ELISA reactions in a BYDV-MAV-specific assay but were negative in RT-PCR expected to detect BYDV-MAV (Malmstrom and Shu 2004) and were also transmitted by *R. padi*. Here we describe the molecular and biological properties of these isolates and also the identity of other virus isolates from barley and oats. A preliminary account of aspects of this work was reported by Al-Mashhadani et al. (2012).

Materials and methods

Virus isolates

On the basis of typical yellow dwarf symptoms of stunting and chlorosis or reddening (Domier 2009), a total of 17 samples of different cereal plants were collected in 2011 from the northern grain belt region of Australia, including a range of locations in southern Qld and northern NSW. In addition, archived isolates of YDVs from oats (2) and barley (2) collected in 2010 from NSW were obtained from the collection at the Tamworth Agricultural Institute, NSW. Leaf tissue of all samples was freeze dried, lodged in the Queensland Department of Primary Industries (DPI) Plant Virus Collection and stored at −20 °C. Furthermore, some freeze-dried archived samples of YDVs from the DPI Plant Virus Collection were used in this study as reference isolates, including the isolates 2109 (CYDV-RPV) from Victoria, 2487 (BYDV-PAV and CYDV-RPV), 2687 and 2688 (both CYDV-RPV), all from Qld, and 2689 (BYDV-PAV) from NSW, Australia (Table 1).

Aphid colonies and transmission tests

Three non-viruliferous YDV aphid vectors species (*R. maidis*, *R. padi* and *M. dirhodum*) were maintained in Brisbane, Qld and were derived from colonies originally maintained at the Tamworth Agricultural Institute, NSW. *R. maidis* colonies were maintained on barley, *R. padi* on barley or oats, and *M. dirhodum* on oats or wheat. To study the aphid specificity of YDV transmission, aphids were allowed an acquisition access period (AAP) of 24–48 h on detached leaves from infected plants. They were then transferred to caged healthy plants of wheat cv. 'Crusader' or oat cv. 'Culgoa' or cv. 'Genie' for an inoculation access period (IAP) of at least 72 h. At the end the inoculation period, the aphids were sprayed with an insecticide (imidacloprid), and the plants checked twice a week for symptoms of yellow dwarf disease. After a period of at least four weeks, the emerging leaves of inoculated plants were tested for infection by DAS-ELISA or RT-PCR as described below.

Serological tests

Samples were tested by double antibody sandwich (DAS) ELISA for BYDV-MAV, BYDV-PAV and CYDV-RPV, essentially as described by the manufacturer (AGDIA Cat. No. 26500, 27500 and 28001, respectively), except that reaction volumes of 50 µL were used for all steps except substrate addition, when 100 µL was used. A_{405nm} values were recorded using a Microtiter Plate Reader (Bioscan EX) and values equal to or exceeding three times the mean of healthy control values were regarded as positive.

RNA extraction and RT-PCR

Total nucleic acid extracts (TNAE) were prepared from fresh (30 mg) or freeze-dried (3 mg) tissue using a BioSprint 15

Table 1 Collection date, source and viruses identified in barley and oat survey samples and reference isolates

DAF Virus collection Isolate no.	Host	Date of collection	Location	Alternative code	Source
2109	oat	2007	Vic		Qld DPI collection archived as CYDV-RPV, BYDV-PAV, CYDV-RMV
2487	barley	12/10/2009	Qld		Qld DPI collection archived as CYDV-RPV, BYDV-PAV, BYDV-MAV
2687	barley	23/09/2010	Junabee, Qld		Qld DPI collection archived as CYDV-RPV
2688	barley	23/09/2010	Junabee, Qld		Qld DPI collection archived as CYDV-RPV
2689	barley	22/09/2010	Tulloona NSW		Qld DPI collection archived as BYDV-PAV
2851	barley	29/07/2011	Warwick, Qld		This study
2852	oat cv. Graza 80	10/08/2010	Boggabri, NSW	4946CMd#1	NSW collection archived as BYDV-PAV
2854	barley cv. Commander	20/09/2010	Tamworth, NSW	5047BRp#1	NSW collection archived as CYDV-RMV-like
2855	barley cv. Gairdner	20/09/2010	Tamworth, NSW	5047DRm#1	NSW collection archived as CYDV-RPV
2856	oat cv. Eurabbie	10/08/2010	Dunedoo, NSW	5023BRp/Rp#2	NSW collection archived as CYDV-RPV
2868	oat	2/08/2011	Warwick, Qld		This study
2869	oat	2/08/2011	Warwick, Qld		This study
2870	oat	2/08/2011	Warwick, Qld		This study
2871	oat	2/08/2011	Warwick, Qld		This study
2872	oat	2/08/2011	Warwick, Qld		This study
2876	oat	12/08/2011	Warwick, Qld		This study
2877	oat	12/08/2011	Warwick, Qld		This study
2884	oat	26/08/2011	Gatton, Qld		This study
2886	barley	29/07/2011	Warwick, Qld		This study
2887	barley	29/07/2011	Warwick, Qld		This study
2888	barley	29/07/2011	Warwick, Qld		This study
3001	oat	17/10/2011	Bellata, NSW		This study
3002	oat	17/10/2011	Bellata, NSW		This study
3003	oat	17/10/2011	Bellata, NSW		This study
3004	oat	10/10/2011	Warwick, Qld		This study

workstation with a BioSprint 15 DNA Plant Kit (QIAGEN) as per the manufacturer's instructions but without the use of RNase A in buffer RPW. Extracts were stored at -20 °C.

Reverse transcription PCR was performed using Invitrogen reagents and essentially following either the Malmstrom and Shu (2004) protocol or that described by Chomic et al. (2010) using primers C1F1 and C1R2. To screen isolates which were positive by ELISA for BYDV-MAV (Table 2) for the viral sequence assembled using high throughput sequencing (see below), primers 2484F (5′-CGTACCTTC AAGGAAACGCC-3′) and 2951R (5′-TGGCCTTCCTCG AGTTGTTC-3′), which span the ORF2-ORF3 junction (a common recombination point for BYDV species (Pagán and Holmes 2010), were designed and used in two step RT-PCR to amplify 476 bp amplicons. Details of these protocols and preparation of amplicons for Sanger sequencing are provided in the Supplementary Materials and Methods.

High-throughput sequencing

RNA was extracted from a ground sample of isolate 2871 (prepared with a Tissue Lyser (QIAGEN)) using a TRIzol™ Plus RNA Purification Kit (Cat. No. 12183555, Thermo Fisher Scientific) as per manufacturer's instructions. A ribodepleted library was prepared using TruSeq Stranded Total RNA with Ribo-Zero Plant kit (Illumina) by AGRF (Melbourne Vic., Australia) and 150 bp pair-ended reads were sequenced using a 300 cycle kit on an Illumina NovaSeq X sequencer.

Raw reads were uploaded to the Galaxy Australia web platform at usegalaxy.org.au (The_Galaxy_Community

Table 2 Summary of properties of yellow Dwarf virus isolates in this study

Isolate	ELISA BYDV-PAV	ELISA CYDV-RPV	ELISA BYDV-MAV	+ve aphid transmission (positive ELISA following inoculation)	-ve aphid transmission	RT-PCR, M&S[A] MAV, PAV, SGV	RT-PCR, M&S[B] GPV, RMV, RPV	RT-PCR, Chomic[C] MAV, PAS, PAV	RT-PCR CrRLV ORF2-3	Amplicons identified
2109	nd	nd	nd	nd	nd	nd	nd	+	nd	BYDV-PAV
2487	-	+	+	nd	nd	nd	nd	nd	+	CrRLV+BYDV-PAS
2689	nd	nd	nd	nd	nd	nd	nd	+	nd	BYDV-PAV
2851	+	-	-	nd	Md[D], Rm[E], Rp[F]	+	-	+	nd	BYDV-PAV
2852	+	-	-	nd	Md	+	-	nd	nd	BYDV-PAV
2854	-	-	-	nd	Rm, Rp	-	+	nd	+	CrRLV+BVG
2855	-	+	-	nd	nd	-	+	nd	nd	CYDV-RPV
2856	-	+	-	Rp (RPV)	nd	-	+	nd	nd	CYDV-RPS
2868	-	+	-	Rp (RPV)	Md, Rm	-	+	nd	nd	CYDV-RPV
2869	-	+	-	Rp (RPV)	nd	-	+	nd	nd	CYDV-RPV
2870	-	+	-	Rp (RPV)	Md, Rm	+	+	nd	nd	CYDV-RPV+BYDV-PAV
2871	-	-	+	Rp (MAV)	Rm	-	-	+	+	CrRLV
2872	-	-	+	nd	nd	-	-	+	+	CrRLV
2876	-	-	+	nd	nd	-	-	+	+	CrRLV
2877	-	-	+	Rp (MAV)	Md, Rm	-	-	+	+	CrRLV
2884	-	-	-	nd	nd	-	+	nd	nd	BVG
2886	nd	nd	nd	nd	nd	-	+	nd	nd	BVG
2887	nd	nd	nd	nd	nd	-	+	nd	nd	BVG
2888	nd	nd	nd	nd	nd	-	+	nd	nd	nd
3001	-	+	+	nd	nd	nd	nd	+	nd	CrRLV
3002	-	-	+	nd	nd	nd	nd	+	nd	CrRLV
3003	+	-	-	nd	nd	nd	nd	nd	nd	nd
3004	+	-	-	nd	nd	nd	nd	nd	nd	nd

[A] Malmstrom and Shu (2004) primers to detect BYDV-MAV, -PAV and -SGV; [B] Malmstrom and Shu (2004) primers to detect CYDV-RPV, MYDV-RMV and WYDV-GPV; [C] Chomic et al. (2010) primers to detect BYDV-MAV, -PAV and -PAS; [D]*Metapolophium dirhodum*; [E]*Rhopalosiphum maidis*; [F]*R. padi*; nd, not determined; + positive reaction; - negative reaction

2024), and the public server was used to analyse the data. Quality control and trimming of reads were conducted using FastQC version 0.72+galaxy1 (Andrews, n.d.) and Trimmomatic version 0.36.6 (Bolger et al. 2014) with an ILLUMINACLIP step to remove standard TruSeq3 adapter sequences, the SLIDING WINDOW operation to ensure average quality over 4 bases of at least 20, and the HEADCROP operation to remove the first 12 bases from each read. MEGAHIT version 1.2.9 (Li et al. 2015) with a kmer length of 27 was used to assemble contigs *de novo*. A megaBLAST analysis was used to identify virus-like contigs using NCBI BLAST+BLASTn (version 2.14.1+galaxy1) and the GenBank Virus RefSeq database (downloaded 10 July 2020). Annotation of the genome was conducted using Geneious Prime (v 2022.2.2, Biomatters Ltd).

Data analysis

Sequences were compared to those held in GenBank, NCBI (http://BLAST.ncbi.nlm.nih.gov/BLAST.cgi) using the BLASTn algorithm (Altschul et al. 1990). Sequence alignments were done in the MEGA X program (Kumar et al. 2018) using the Muscle algorithm and maximum likelihood phylogenetic trees were constructed using complete coat protein coding sequences and near-complete genome nucleotide sequence of isolate 2871 and selected YDV reference sequences. Bootstrap confidence limits were derived from 100 replicates.

Virus acronyms and GenBank reference sequence accession numbers are provided in the Supplementary Materials and Methods.

Fig. 1 A and B, Symptoms caused by cereal red leaf virus in oats at Warwick, Qld

Results

Serological assays

The results of DAS-ELISA assays of the cereal isolates collected from Qld and NSW are summarised in Table 2 (details in Supplementary Table 1). Of 18 isolates assayed, four samples tested positive for BYDV-PAV from Qld (2) and NSW (2), seven samples with strong leaf reddening (Fig. 1) tested positive for BYDV-MAV from Qld (5) and NSW (2), and seven samples tested positive for CYDV-RPV from Qld (4) and NSW (3). Two isolates were shown by ELISA to have mixed infection: isolate 2487 from Qld and isolate 3001 from NSW both tested positive for CYDV-RPV and BYDV-MAV.

Aphid transmission

Aphid transmission tests were conducted for nine of the isolates which tested positive by DAS-ELISA or RT-PCR. The results of DAS-ELISAs on the plants inoculated using the three tested cereal aphid vectors of YDVs are summarised in Table 2 (details in Supplementary Table 2).

YDVs were successfully transmitted by only the aphid species *R. padi* from isolates 2856, 2868 and 2869 (CYDV-RPV positive by DAS-ELISA), 2870 (positive for CYDV-RPV by DAS-ELISA and BYDV-PAV by RT-PCR, see below) and isolates 2871 and 2877 (BYDV-MAV positive by DAS-ELISA). YDV-like symptoms were observed on most of the inoculated plant leaves except for isolate 2871. *R. padi* was unable to transmit BYDV-PAV from isolate 2851.

No transmission occurred when the species *M. dirhodum* was used in transmission tests with the isolates 2851 and 2852 (BYDV-PAV positive by DAS-ELISA), 2868 (CYDV-RPV positive by DAS-ELISA), 2870 (positive for CYDV-RPV by DAS-ELISA and BYDV-PAV by RT-PCR) and 2877 (BYDV-MAV positive by DAS-ELISA), and no clear symptoms of yellow dwarf disease were exhibited by the

inoculated plants. Similarly, no transmission of any isolate was obtained using the aphid *R. maidis*.

RT-PCR and sequence analysis of amplicons

Virus isolates that were positive for BYDV-PAV and CYDV-RPV by DAS-ELISA, where tested, were amplified using the primers Yan-R, Shu-F, S2a-F and S2b-F following the Malmstrom and Shu (2004) protocol (Table 2). These primers produced an 830 bp amplicon from tissue infected with BYDV-PAV, and a 372 bp amplicon from tissue infected with CYDV-RPV (Fig. 2). As reported by Malmstrom and Shu (2004), 300 bp fragments that are non-specific amplicons were produced in some samples (Fig. 2, lane 1, 2, 9 and 12). In addition, using the same multiplex RT-PCR system, 372 bp fragments were produced from six of the isolates that were negative by DAS-ELISA (2884, 2885, 2886, 2887, 2888 and 2854; not shown). However, these amplicons appeared feint on the 1% agarose gel, and therefore, these and some additional isolates which gave feint bands (2856 and 2868) were re-amplified following the same protocol and stronger bands were produced for direct sequencing.

The BYDV-MAV-like isolates from oat from Qld were only amplified after following the Chomic et al. (2010) protocol, and not by the basic multiplex RT-PCR system of Malmstrom and Shu (2004) (Figs. 2 and 5). The two primers (C1F1, C1R2) used to amplify these isolates produced 156 bp fragments (Fig. 3). The BYDV-MAV-like isolates were distinct from all other recognised YDVs and the nearest match was to a near complete sequence of the coat protein gene of a luteovirus from *Hierochloe* sp. from New Zealand (EF408185.1), which shared 95.3% nucleotide identity with the amplicon sequence from isolate 2871.

To further examine samples for the presence of BYDV-MAV-like isolates, the sequencing primers 2484F and 2951R were used, and resulted in the detection of two additional isolates from barley (Table 2). Interestingly, a second, smaller amplicon, derived from BYDV-PAS, was generated with these primers from isolate 2487. The primers were designed as sequencing rather than virus-specific primers and upon examination, BYDV-PAS had only two mismatches with primer 2484F (3′ nts 3 and 6) and primer 2951R had a potential alternative binding site with an exact match for the first ten 3′ nt. The potential 195 bp amplicon matched the BYDV-PAS sequence obtained.

The GenBank accession codes for the sequenced isolates are listed in Table 3 as are the closest GenBank match for each of the amplicons sequenced. The viruses detected in this study from oat included CYDV-RPS from NSW, CYDV-RPV and BYDV-PAV from both NSW and Qld, and additionally BVG in Qld. From barley, CYDV-RPV was detected in NSW, BYDV-PAV and BYDV-PAS from Qld, and BVG from both NSW and Qld.

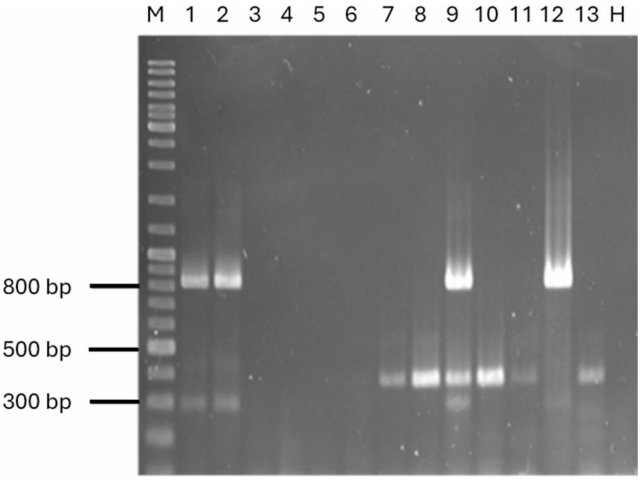

Fig. 2 PCR products for the DAS-ELISA positive isolates following the Malmstrom and Shu (2004) protocol. The expected product sizes are 830 bp for BYDV-PAV, -MAV and -SGV, and 370 bp for CYDV-RPV, -RMV and -GPV. Lanes 1, 2 and 12, BYDV-PAV isolates (2851, 2852 and 2689); 3–6, BYDV-MAV-like isolates (2871, 2872, 2876 and 2877); 7, 8, 10, and 13, BYDV-RPV (2868, 2869, 2855, and 2688); 9, BYDV-PAV and -RPV isolate (mixed infection; 2870); 11, CYDV-RPS; H, healthy wheat control; M, Fermentas GeneRuler DNA marker ladder (SM0331)

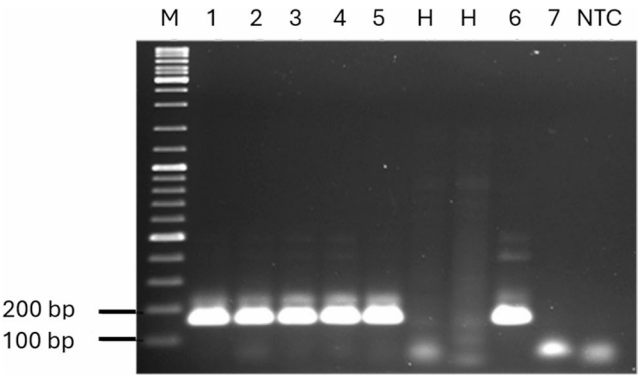

Fig. 3 PCR products for BYDV-MAV-like isolates following Chomic et al. (2010) protocol. The expected product size is 156 bp for BYDV-PAV, -MAV and -SGV. Lanes 1–5, isolates 2871, 2872, 2876, 2877 and 2877 repeated; H, healthy oat control; 6, BYDV-PAV positive control (2851); 7, BYDV-RPV negative control (2868); NTC, no template control; M, Fermentas GeneRuler DNA marker ladder (SM0331)

Sequence analysis of isolate 2871

Following *de novo* assembly, a virus-like contig of 5,650 nucleotides was identified from a BLASTn analysis against the NCBI Virus RefSeq database (10 July 2020). This sequence (GenBank accession PX666645) represents a near complete genome with an organization typical of a member

Table 3 Closest GenBank matches from BLASTn searches using partial genomic sequences of YDV isolates from this study

Isolate	Amplicon sequence length (nt)	GenBank accession number	Closest BLASTn match		Nucleotide identity (%)
			Virus	GenBank accession	
2109	82[a]	See Supp. Data	BYDV-PAV	MN648441.1	98.8
2487	136[b]	PX666646	BYDV-PAS	MN128938.1	100
2487	399[c]	PX666647	BYDV-OYV	MK012649	86.9
2689	101[a]	See Supp. Data	BYDV-PAV	D85783.1	99.0
2851	742[d]	PX666648	BYDV-PAV	EF408160.1	98.5
2852	731[d]	PX666649	BYDV-PAV	EF521840.1	99.2
2854	286[d]	PX666651	BVG	MK103387.1	100
2854	399[c]	PX666650	BYDV-PAV	KT252978.1	83.2
2855	278[d]	PX666652	CYDV-RPV	GU002338.1	97.8
2856	334[d]	PX666653	CYDV-RPS	MK975889.1	98.8
2868	276[d]	PX666654	CYDV-RPV	KY553235.1	96.4
2869	334[d]	PX666655	CYDV-RPV	KY553235.1	97.0
2870	325[d]	PX666656	CYDV-RPV	KY553235.1	98.8
2870	754[d]	PX666657	BYDV-PAV	EF408160.1	98.4
2871	95[a]	See Supp. Data	Hierochloe luteovirus	EF408185.1	94.7
2871	428[dC]	PX666658	BYDV-PAV	KT252978.1	82.3
2872	84[a]	See Supp. Data	Hierochloe luteovirus	EF408185.1	95.1
2872	399[c]	PX666659	BYDV-PAS	OR771728.1	83.4
2876	84[a]	See Supp. Data	Hierochloe luteovirus	EF408185.1	95.2
2876	399[c]	PX666660	BYDV-PAS	OR771728.1	83.4
2877	112[a]	PX666661	Hierochloe luteovirus	EF408185.1	95.5
2877	428[c]	PX666662	BYDV-PAV	KT252978.1	82.3
2884	334[d]	PX666663	BVG	ON419455.1	99.1
2886	334[d]	PX666664	BVG	ON419455.1	99.1
2887	313[d]	PX666665	BVG	ON419455.1	98.7
3001	112[a]	PX666666	Hierochloe luteovirus	EF408185.1	95.5
3002	112[a]	PX666667	Hierochloe luteovirus	EF408185.1	95.5

[a] Primers C1F1 and C1R2 reported as giving a 156 bp amplicon with BYDV-PAV, BYDV-PAS and BYDV-MAV (Chomic et al. 2010)

[b] Primers 2484F and 2951R sequencing primer - non-target amplicon

[c] Primers 2484F and 2951R sequencing primer - target amplicon

[d] Primers Yan-R, Shu-F, S2a-F and S2b-F, reported as giving an 830 bp amplicon with BYDV-PAV, BYDV-MAV, or BYDV-SGV and a 372 bp amplicon with CYDV-RPV, CYDV-GPV or BYDV-RMV (Malmstrom and Shu 2004)

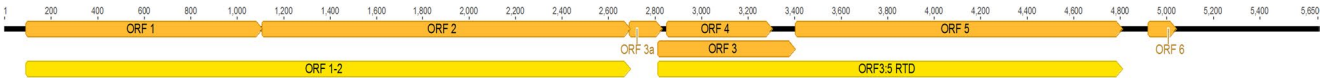

Fig. 4 Genome organization of cereal red leaf virus isolate 2871

of the genus *Luteovirus* (family *Tombusviridae*) including open reading frames (ORFs) 1, 2, 3, 3a, 4, 5 RTD and 6 (Miller and Lozier 2022) (Fig. 5). ORF 1 encodes a putative replication associated protein and via a -1 frameshift encodes an extended product ORF 1–2, incorporating the RdRp of ORF 2. ORF 3 (coat protein) has a read through domain which results in an extended ORF 3 + 5 protein. The recently recognised ORF 3a (long distance movement protein) and a putative ORF 6 (unknown function) are also present. In a phylogenetic analysis of the nucleotide sequences of both the complete CP coding gene and the complete genome, isolate 2871 was located on a separate branch to all other YDVs (Figs. 5 and 6). The position of the ORFs, size of the encoded proteins, and their nearest GenBank matches are shown in Table 4. The nearest luteovirus whole reference genome match was 64.0% at the nucleotide level to BYDV-GAV (NC_004666) (Supplementary Table 3).

Discussion

The diversity of recognised YDVs in the *Poaceae* has increased markedly in recent years. When first closely investigated in the 1980s (Greber 1988; Guy et al. 1986, 1987; Johnstone 1995; McKirdy and Jones 1993; Sward & Lister 1988; Waterhouse and Helms 1985) only four YDV

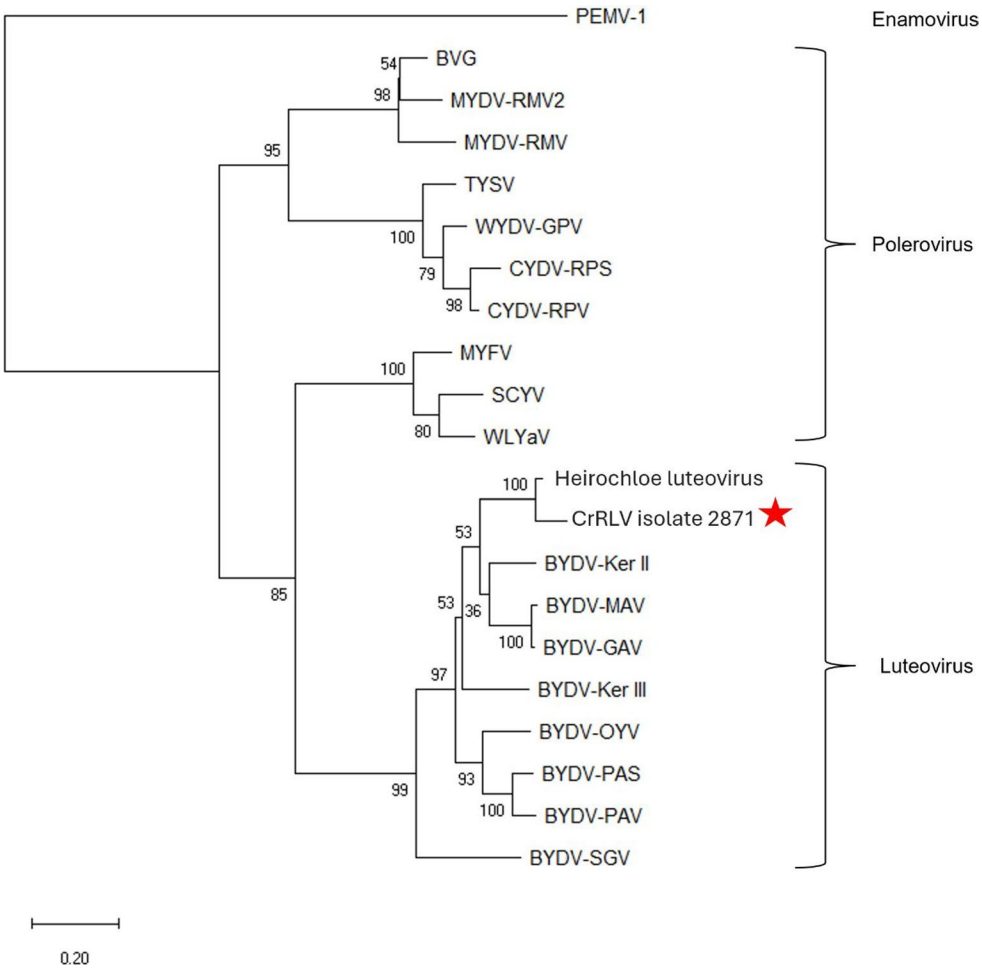

Fig. 5 Maximum likelihood tree based on a Muscle alignment (MEGA X) of the nucleotide sequences of the complete coat protein coding sequences of CrRLV isolate 2871, Heirochloe luteovirus, selected yellow dwarf viruses from the genera *Luteovirus* and *Polerovirus* and using PEMV as an outgroup. CrRLV isolate 2871 (red star); See Methods section for virus acronyms

species were identified in Australia, based on vector specificity and serology. Interestingly, two of these may have been mis-identified as discussed below. Recent detections of BVG (Nancarrow et al. 2019), BYDV-PAS (Nancarrow et al. 2024b) and a virus most closely related to CYDV-RPV (Nancarrow et al., 2024a) have brought the total reported YDVs from Australia to seven. In the present study, using field samples collected in 2011 and some archived samples from 2007 to 2010, the known geographical distribution of some YDVs in Australia was extended. BYDV-PAS and CYDV-RPV were found in Qld for the first time, CYDV-RPS in NSW for the first time and BVG in NSW and Qld for the first time.

Interestingly, an archived YDV sample (isolate 2854) in this work, previously identified as MYDV-RMV based on vector specificity, was revealed to be BVG by sequence analysis. To the best of our knowledge all previous records of MYDV-RMV from Australia were based on vector specificity and/or serological testing, and no sequence data are available. This raises the possibility that they were potentially misidentifications and that there are no definitive records of MYDV-RMV from Australia. Similarly, an archived sample (isolate 2856) previously identified as CYDV-RPV serologically and with transmission by *R. padi* was shown through sequence analysis to be CYDV-RPS. These two viruses are known to cross-react serologically (Nancarrow et al. 2023) and to share a common aphid vector (*R. padi*), complicating accurate identification in the absence of sequence data.

Similarly, all previous identifications of BYDV-MAV from Australia were confirmed by ELISA and interestingly, vector transmission was unexpectedly efficient with *R. padi* (Sward & Lister 1988). *Sitobion avenae*, the generally recognised primary vector of BYDV-MAV, has not been recorded from Australia. Also, there are no sequence data available from previous putative BYDV-MAV isolates from Australia. In the present study, all YDV isolates that gave a positive ELISA reaction with BYDV-MAV antibodies were in fact infected with a novel luteovirus for which we propose the name cereal red leaf virus (CrRLV, tentative scientific name *Luteovirus foliarubra)*). CrRLV isolate 2871, for which a near complete genome sequence was obtained, was present in an oat plant displaying symptoms of yellow/reddening and stunting, and no additional viruses were detected in this

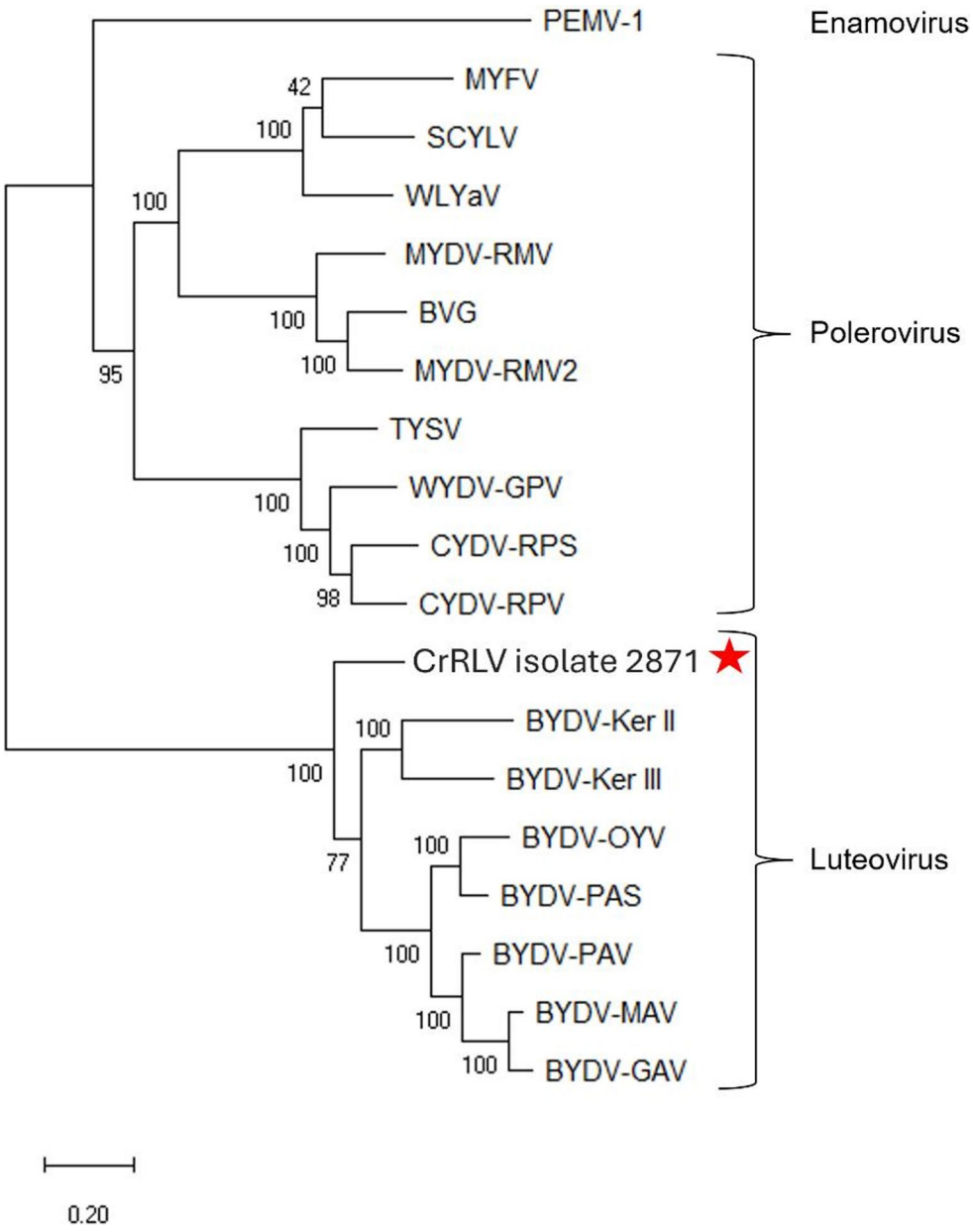

Fig. 6 Maximum likelihood tree based on a Muscle alignment (MEGA X) of the nucleotide sequences of the complete genomes of CrRLV isolate 2871 and recognized yellow dwarf viruses from the genera *Luteovirus* and *Polerovirus* and using PEMV as an outgroup. See Methods section for virus acronyms

Table 4 Size and position of open reading frames of oat yellow Dwarf virus isolate 2871

ORF	Protein size (kDa)	Nt position on CrRLV genome	BLASTp closest match	Source accession	% amino acid identity
1	38.89	91–1110	BYDV-PAV	KU170668.1	63.72
2	60.42	1107–2693	BYDV-KerIII	NC_043123.1	81.47
3	21.79	2811–3401	Hierochloe luteovirus	EF408185.1	87.11
3a	5.28	2687–2830	BYDV-PAS	PP405116.1	95.56
4	16.80	2848–3303	Hierochloe luteovirus	EF408185.1	87.42
3RTD	73.57	2687–4811	BYDV-MAV	OQ686672.1	57.44
5		3402–4811	BYDV-PAV	EF521833.1	55.60
6	4.06	4921–5043	No identified matches	n/a	n/a

plant by *de novo* assembly of HTS data. These isolates were transmitted efficiently by *R. padi* and although cross-reacting with BYDV-MAV antibodies, share only 69% aa identity in the CP. It is also likely, therefore, that previous records of BYDV-MAV were actually of CrRLV and that there are also no definitive records of BYDV-MAV from Australia. CrRLV was recorded from both NSW and Qld in oats and barley.

In the present work, not all expected aphid transmissions were achieved and there are several possible reasons. In most experiments, limited numbers of test plants and aphids were used, possibly resulting in the failure to detect lower rates of transmission. In some cases, e.g. for BYDV-PAV in a mixed infected with CYDV-RPV in isolate 2870, low virus titre may have been the cause; BYDV-PAV was detected by RT-PCR, but not by ELISA in this sample. It is also possible that viruses detected in the original field samples were not present in the tillers later transferred to Brisbane for testing. Therefore, while there can be confidence in the instances of confirmed aphid transmission, there could be a degree of uncertainty surrounding negative transmissions.

CrRLV has a genome organisation typical of the genus *Luteovirus* and should be considered a unique species, according to the luteovirus species demarcation guidelines of the International Committee on Taxonomy of Viruses (Domier 2009). It has greater than 10% aa sequence difference in all ORFs except ORF 3a (95.6% aa identity with BYDV-PAS; PP405116.1). The next closest matches for the other ORFs range from about 56–87%. During this study, a partial luteovirus sequence (GenBank EF408185.1) from the New Zealand native grass *Hierochloe* (syn. *Anthoxanthum*) *redolens* was lodged, which shared 87% aa identity in the CP and was also phylogenetically closely related to CrRLV. It was also not possible to amplify this isolate in PCR with the Malmstrom and Shu (2004) primers (Catia Delmiglio, personal communication). It may represent a strain of CrRLV or a closely related virus but unfortunately this isolate is no longer available for comparison. During the preparation of this manuscript, we also became aware of the more recent detection of CrRLV in Victoria (GenBank LC884787-LC884793).

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Declarations

Ethics approval Not applicable.

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

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