Supplementary Materials and Methods

Reverse transcription and PCR

Reverse transcription was performed using Invitrogen reagents and essentially following the Malmstrom and Shu (2004) protocol. Initially, 2 µL Yan-R (10 µM) reverse primer and 3 µL of TNAE were mixed with 8 µL RNase-free water, heated at 80°C for 10 min, and the reaction then cooled on ice followed by brief centrifugation. The following reagents were then added: 4 µL of 5 × First-Strand buffer, 2 µL of 0.1 M DTT, 0.5 µL of 0.6 µg/ µL BSA and 0.5 µL of SuperScript III (200 U/µL). The reaction was incubated at 55°C for 45 min, then 70°C for 10 min, chilled on ice and then centrifuged briefly.

PCR reactions were also performed using Invitrogen reagents following either of two protocols. The first protocol was the basic multiplex RT-PCR system essentially as described by Malmstrom and Shu (2004) except that the total reaction volume was 25 µL and included 1.5 µL of cDNA and 1 U of *Taq* DNA polymerase (Cat. No. 10-342-020). The thermocycling conditions were 95°C for 1 min, then 35 cycles of 95°C for 20 s, 60°C or 55°C for 20 s and 72°C for 30 s, followed by 72°C for 5 min. The second protocol was based on that described by Chomic et al. (2010) using primers C1F1 and C1R2. The total reaction volume was 20 µL and consisted of 1 × PCR buffer, 1 mM $MgCl_2$, 200 µM dNTPs, 490 µM primer C1F1 and 400 µM primer C1R2, 1 U *Taq* DNA polymerase, and 1 µL of cDNA. The thermocycling conditions were 95°C for 1 min, then 40 cycles of 95°C for 20 s, 50°C for 30 s and 72°C for 30 s, followed by 72°C for 7 min.

To amplify viral sequence across the ORF2-ORF3 junction for isolates positive by ELISA for BYDV-MAV with PCR primers 2484F (5′-CGTACCTTCAAGGAAACGCC-3′) and 2951R (5′-TGGCCTTCCTCGAGTTGTTC-3′), reverse transcription was conducted with the following modifications to the above method: 0.25 µL of RNaseOUT (40 U/ µL) was used in place of BSA, SuperScript III (200 U/ µL) was reduced to 0.25 µL and reactions were incubated at 50°C instead of 55°C. For PCR, the reactions consisted of 1 × PCR buffer, 2 mM $MgCl_2$, 200 µM dNTPs, 400 µM each primer, 1.5 U *Taq* DNA polymerase and 2 µL of cDNA, and thermocycling conditions were 94°C for 1 min, then 35 cycles of 94°C for 10 s, 57°C for 10 s and 72°C for 30 s, followed by 72°C for 3 min.

All PCR products were analysed by electrophoresis on a 1% or 1.5% agarose gel in 0.5 × TBE buffer by comparison with 200 ng of a DNA marker (Fermentas GeneRuler DNA ladder, Cat.

No. SM0331), post-stained with ethidium bromide and visualized using a Bio-Rad gel documentation system under UV illumination.

Sanger sequencing

For purification prior to sequencing, PCR products from the reactions with Malmstrom and Shu (2004) and Chomic et al. (2010) primers were electrophoresed using an E-gel Clone Well 0.8% SYBR Safe gel (Invitrogen) on an iBase (Ethrong Biotechnologies Ltd) according to the manufacturer's instructions and were then extracted in water. Excised target amplicons of electrophoretically separated PCR products generated using the primers designed in this study were purified using the ISOLATE II PCR and Gel Kit (Meridian Bioscience). PCR products were then sent to the Australian Genome Research Facility (AGRF, Brisbane Qld, Australia) for direct sequencing using the Sanger method using single primers from the reaction that generated that amplicon.

Virus acronyms and GenBank accession reference sequences

BVG, NC_029906 barley virus G; BYDV-GAV, NC_004666 barley yellow dwarf virus-GAV; BYDV-Ker-II, NC_021481 barley yellow dwarf virus Ker-II; BYDV-Ker III, NC_043123 barley yellow dwarf virus Ker-III; BYDV-MAV, NC_003680 barley yellow dwarf virus-MAV; BYDV-OYV, MK012645 BYDV-OYV (oat yellowing virus); BYDV-PAS, NC_002160 barley yellow dwarf virus-PAS; BYDV-PAV, NC_004750 barley yellow dwarf virus PAV; BYDV-SGV, NC_043124 barley yellow dwarf virus-SGV; CYDV-RPS, NC_002198 cereal yellow dwarf virus-RPS; CYDV-RPV, LC765997 cereal yellow dwarf virus RPV; CYDV-RPV, NC_004751 cereal yellow dwarf virus-RPV; MYDV-RMV, NC_021484 maize yellow dwarf virus-RMV; MYDV-RMV2, NC_029990 maize yellow dwarf virus-RMV2; PEMV-1, NC_003629 pea enation mosaic virus-1; MYFV, MT520166 miscanthus yellow fleck virus; SCYLV, NC_000874 sugarcane yellow leaf virus; TYSV, OM829809 triticum yellow stripe virus; WYDV-GPV, NC_012931 wheat yellow dwarf virus-GPV; and WLYaV, NC_035451 wheat leaf yellowing associated virus.

58 **Supplementary Table 1.** Reaction of survey samples in DAS-ELISA against antisera to
59 BYDV-PAV, BYDV-MAV and CYDV-RPV.

Isolate	BYDV-PAV [A]	BYDV-MAV [A]	CYDV-RPV [A]
2851	**0.686 / 0.006**	0.030 / 0.079	0.008 / 0.002
2852	**0.820 / 0.006**	0.039 / 0.044	0.003 / 0.004
2854	0.009 / 0.006	0.036 / 0.044	0.003 / 0.004
2855	0.008 / 0.006	0.039 / 0.044	**0.616 / 0.004**
2856	0.008 / 0.006	0.039 / 0.044	**1.628 / 0.004**
2868	0.011 / 0.006	0.066 / 0.044	**1.277 / 0.004**
2869	0.010 / 0.006	0.044 / 0.044	**0.199 / 0.004**
2870	0.008 / 0.006	0.037 / 0.044	**2.222 / 0.004**
2871	0.006 / 0.006	**0.300 / 0.044**	0.013 / 0.004
2872	0.009 / 0.006	**0.244 / 0.044**	0.003 / 0.004
2876	0.017 / 0.021	**0.209 / 0.023**	0.022 / 0.013
2877	0.014 / 0.021	**0.239 / 0.023**	0.012 / 0.013
2884	0.009 / 0.010	0.041 / 0.035	0.036 / 0.028
3001	0.010 / 0.011	**0.054 / 0.013**	**2.325 / 0.023**
3002	0.018 / 0.011	**0.055 / 0.013**	0.041 / 0.023
3003	**0.264 / 0.011**	0.009 / 0.013	0.031 / 0.023
3004	**0.355 / 0.021**	0.016 / 0. 013	0.010 / 0.007

60 [A] Mean A_{404nm} test sample / appropriate healthy control

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Supplementary Table 2. Aphid transmission tests with selected YDV isolates.

Isolate	YDV species[A]	Test plant	Aphid species	Total number of plants/total number of aphids	Virus species indicated by ELISA in test plants		
					PAV	MAV	RPV
2851	BYDV-PAV	wheat cv. Crusader	*M. dirhodum*	1/20	-	-	-
2851	BYDV-PAV	oat cv. Culgoa	*R. maidis*	8/20	-	-	-
2851	BYDV-PAV	oat cv. Genie	*R. padi*	8/20	-	-	-
2852	BYDV-PAV	wheat cv. Crusader	*M. dirhodum*	1/20	-	-	-
2854 [B]	BVG + CrRLV	oat cv. Genie and Culgoa	*R. maidis*	4/20	-	-	-
2854 [B]	BVG + CrRLV	oat cv. Genie	*R. padi*	8/20	-	-	-
2856	CYDV-RPS	wheat cv. Crusader	*R. padi*	1/8	-	-	+
2868	CYDV-RPV	oat cv. Culgoa	*M. dirhodum*	8/20	-	-	-
2868	CYDV-RPV	oat cv. Genie	*R. maidis*	8/20	-	-	-
2868	CYDV-RPV	oat cv. Genie and Culgoa	*R. padi*	5/25	-	-	+
2869	CYDV-RPV	oat cv. Genie and Culgoa	*R. padi*	8/40	-	-	+
2870 [C]	BYDV-PAV + CYDV-RPV	oat cv. Culgoa	*M. dirhodum*	8/20	-	-	-
2870 [C]	BYDV-PAV + CYDV-RPV	oat	*R. maidis*	8/20	-	-	-
2870 [C]	BYDV-PAV + CYDV-RPV	oat cv. Genie and Culgoa	*R. padi*	4/20	-	-	+
2871	CrRLV	oats	*R. maidis*	8/20	-	-	-
2871	CrRLV	oat cv. Genie and Culgoa	*R. padi*	4/22	-	+	-
2877	CrRLV	oat cv. Genie	*M. dirhodum*	8/20	-	-	-
2877	CrRLV	oat cv. Genie	*R. maidis*	8/20	-	-	-
2877	CrRLV	oat cv. Genie and Culgoa	*R. padi*	5/28	-	+	-

[A]Virus species determined by sequencing of PCR amplicons except for CYDV-RPV in isolate 2870 which was determined by ELISA only.

[B] This isolate was negative in ELISA and thus may not have been infectious for the transmission tests.

[C] This isolate was negative for BYDV-PAV by ELISA and thus may not have been infectious for the transmission tests.

Supplementary Table 3. Percentage nucleotide identities derived from complete genome alignments (Geneious/Muscle) of CrRLV isolate 2871 and selected isolates of other yellow dwarf viruses.

	CrRLV	BYDV-GAV	BYDV-PAS	BYDV-PAV	BYDV-OYL	BYDV-MAV	BYDV-Ker II	BYDV-Ker III	WYDV-GPV	CYDV-RPV	CYDV-RPS	TYSV	WYLaV	SCYLV	MYFV	BVG	MYDV-RMV2	MYDV-RMV	PEMV-1
CrRLV		63.8	63.3	62.7	62.1	60.1	59.8	53.2	27.9	27.7	27.5	26.9	26.5	25.9	25.6	25.6	25.6	25.3	20.1
BYDV-GAV	63.8		71.2	79.4	70.1	84.3	61.4	52.0	29.8	28.9	29.3	28.7	26.8	26.3	26.7	27.1	27.3	26.9	20.6
BYDV-PAS	63.3	71.2		79.1	82.7	66.2	60.2	52.5	30.0	29.6	29.7	28.7	27.3	26.8	26.7	27.0	27.0	26.9	21.1
BYDV-PAV	62.7	79.4	79.1		76.0	74.3	60.6	52.8	30.7	29.8	30.2	29.7	27.5	27.3	27.5	27.3	27.6	27.6	21.1
BYDV-OYV	62.1	70.1	82.7	76.0		65.5	60.2	52.7	29.3	29.1	29.6	28.7	27.4	26.5	26.7	26.8	27.0	26.8	21.3
BYDV-MAV	60.1	84.3	66.2	74.3	65.5		58.3	56.4	30.4	29.4	29.8	29.4	27.2	26.6	26.8	27.6	27.8	27.4	20.9
BYDV-Ker II	59.8	61.4	60.2	60.6	60.2	58.3		54.2	27.0	26.7	27.0	26.2	25.7	25.6	25.6	24.9	25.3	25.2	19.9
BYDV-Ker III	53.2	52	52.5	52.8	52.7	56.4	54.2		28.1	27.9	27.3	27.9	26.0	25.8	25.4	26.4	26.4	26.7	20.9
WYDV-GPV	27.9	29.8	30.0	30.7	29.3	30.4	27.0	28.1		71.1	74.9	67.0	42.5	40.8	41.0	43.4	42.9	42.4	37.1
CYDV-RPV	27.7	28.9	29.6	29.8	29.1	29.4	26.7	27.9	71.1		72.9	68.1	42.7	41.6	41.0	43.8	43.4	42.9	36.7
CYDV-RPS	27.5	29.3	29.7	30.2	29.6	29.8	27.0	27.3	74.9	72.9		65.8	42.6	41.0	41.1	43.2	43.2	42.6	37.4
TYSV	26.9	28.7	28.7	29.7	28.7	29.4	26.2	27.9	67.0	68.1	65.8		42.1	41.0	40.0	42.5	42.4	41.9	35.4
WYLaV	26.5	26.8	27.3	27.5	27.4	27.2	25.7	26.0	42.5	42.7	42.6	42.1		64.9	60.6	47.1	47.8	46.8	33.1
SCYLV	25.9	26.3	26.8	27.3	26.5	26.6	25.6	25.8	40.8	41.6	41.0	41.0	64.9		62.3	45.9	45.9	45.4	31.5
MYFV166	25.6	26.7	26.7	27.5	26.7	26.8	25.6	25.4	41.0	41.0	41.1	40.0	60.6	62.3		45.8	45.9	44.7	31.2
BVG	25.6	27.1	27.0	27.3	26.8	27.6	24.9	26.4	43.4	43.8	43.2	42.5	47.1	45.9	45.8		76.0	69.9	33.1
MYDV-RMV2	25.6	27.3	27.0	27.6	27.0	27.8	25.3	26.4	42.9	43.4	43.2	42.4	47.8	45.9	45.9	76.0		69.7	33.4
MYDV-RMV	25.3	26.9	26.9	27.6	26.8	27.4	25.2	26.7	42.4	42.9	42.6	41.9	46.8	45.4	44.7	69.9	69.7		33.6
PEMV-1	20.1	20.6	21.1	21.1	21.3	20.9	19.9	20.9	37.1	36.7	37.4	35.4	33.1	31.5	31.2	33.1	33.4	33.6	

See Supplementary Materials and Methods section for virus acronyms.

Supplementary Data

Amplicon sequence data too short for GenBank lodgement.

>isolate_2109 [organism=Luteovirus pavhordei] [collector=M. Aftab] coat protein gene, partial cds

ACGGATACTCAAGTCCTACCATCGTTACAAGATCACAAGTATCCGAGTTGAGTTTA
AGTCACACGCGTCCGCCACTACGGCA

>isolate_2689 [organism=Luteovirus pavhordei] [collector= G. Nixon] coat protein gene, partial cds

ATACTTAAGTCCTACCACCGTTACAAGATCACAAGTATCCGTGTTGAGTTTAAGTC
ACACGCGTCCGCAACCACGGCCGGCGCTATCTTTATTGAAC

>isolate_2871 [organism=Cereal red leaf virus] [collector=G. Platz] coat protein gene, partial cds

CTGCGGTATCAAACGGAGTACTTAAGTCCTACCACCGTTACAAGATCACAAGTATT
ACGGTCGAGTTTAAGTCCCACGCCTCAGCAACCACAACC

>isolate_2872 [organism=Cereal red leaf virus] [collector=G. Platz] coat protein gene, partial cds

AACGGAGTACTTAAGTCCTACCACCGTTACAAGATCACAAGTATTACGGTCGAGTT
TAAGTCCCACGCCTCAGCAACCACAACC

>isolate_2876 [organism=Cereal red leaf virus] [collector= M. Sharman] coat protein gene, partial cds

AACGGAGTACTTAAGTCCTACCACCGTTACAAGATCACAAGTATTACGGTCGAGTT
TAAGTCCCACGCCTCAGCAACCACAACC