European Journal of Plant Pathology (2005) 113:321–326
DOI 10.1007/s10658-005-1231-y

Short communication

Relationships of *Barley yellow dwarf virus*-PAV and *Cereal yellow dwarf virus*-RPV from Iran with viruses of the family *Luteoviridae*

M. Rastgou[1,3], B. Khatabi[1], A. Kvarnheden[2] and K. Izadpanah[1]
[1]*Department of Plant Protection, Centre of Excellence for Plant Virology, College of Agriculture, Shiraz University, Shiraz, Iran (Phone: +98 (712) 2653313; Fax: +98 (712) 6280913; E-mail: Bkhatabi@yahoo.com);* [2]*Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [3]*Department of Plant Protection, Collage of Agriculture, Urmia University, Urmia, Iran*

Accepted 15 July 2005

Key words: Luteovirus, *Polerovirus*, *Rhopalosiphum padi*, wheat

Abstract

Barley yellow dwarf disease is one of the most important problems confronting cereal production in Iran. *Barley yellow dwarf virus*-PAV (BYDV-PAV) and *Cereal yellow dwarf virus*-RPV (CYDV-RPV) are the predominant viruses associated with the disease. One isolate of BYDV-PAV from wheat (PAV-IR) and one isolate of CYDV-RPV from barley (RPV-IR) were selected for molecular characterisations. A genome segment of each isolate was amplified by PCR. The PAV-IR fragment (1264 nt) covered a region containing partial genes for coat protein (CP), read through protein (RTP) and movement protein (MP). PAV-IR showed a high sequence identity to PAV isolates from USA, France and Japan (96–97%). In a phylogenetic analysis it was placed into PAV group I together with PAV isolates from barley and oats. The fragment of RPV-IR (719 nt) contained partial genes for CP, RTP and MP. The sequence information confirmed its identity as CYDV. However, RPV-IR showed 90–91% identity with both RPV and *Cereal yellow dwarf virus*-RPS (CYDV-RPS). Phylogenetic analyses suggested that it was more closely related to RPS. These data comprise the first attempt to characterise BYD-causing viruses in Iran and southwest Asia.

Barley yellow dwarf disease (BYD) is one of the economically most important viral diseases of cereals worldwide (D'Arcy and Burnett, 1995). It may cause significant yield losses in major cereal crops like wheat, barley, rice, maize, oat and ryegrass. BYD is caused by a complex of viruses in the family *Luteoviridae*. The most common of these viruses are *Barley yellow dwarf virus*-PAV (BYDV-PAV) and *Barley yellow dwarf virus*-MAV (BYDV-MAV) in the genus *Luteovirus*, and *Cereal yellow dwarf virus*-RPV (CYDV-RPV) in the genus *Polerovirus* (D'Arcy et al., 1999, 2000). The genome of these consists of a positive sense single-stranded RNA molecule with six open reading frames (ORFs) (Miller et al., 1988, 2002). ORF3 encodes the major coat protein (CP) of about 22 kDa, ORF4 encodes a 17 kDa movement protein (MP), and ORF5 is translated with ORF3 to form the read through protein (RTP), which is needed for aphid transmission (Chay et al., 1996). The viruses are vectored by at least 25 aphid species in a persistent (circulative) manner and are limited to the phloem tissues of infected plants (D'Arcy and Burnett, 1995). Many isolates of BYDV/CYDV have been cloned and sequenced (especially the CP region) from all over the world, which facilitates design of primers for virus detection, finding the correlation between amino

The nucleotide sequence data reported appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers AY450425 and AY450454

acid residues and biological characters, a better understanding of the complex epidemiology of the viruses, designing resistant transgenic plants and other means of control (Miller et al., 2002). Recently, BYDV-PAV was divided into two separate species, BYDV-PAV and *Barley yellow dwarf virus*-PAS (BYDV-PAS), based on the nucleotide sequence of the CP gene (Mayo, 2002). In addition, the isolates of BYDV-PAV could be separated into two major clusters (Bisnieks et al., 2004).

BYD was first reported in Iran in 1988, and BYDV-PAV was found to be the predominant serotype of the BYD-causing viruses on both cultivated and non-cultivated cereals in all regions (Rastgou et al., 2004b). In order of decreasing prevalence the following viruses were also detected: CYDV-RPV, BYDV-MAV and Rhopalosiphum maidis virus (RMV). Among the aphids transmitting these viruses in Iran, *Rhopalosiphum padi* was the predominant species followed by *Schizaphis graminum*, *R. maidis* and *Sitobion avenae* (Khatabi et al., 2004b). Because of the importance and prevalence of BYD in Iran and the absence of molecular information on causal viruses, molecular characterisation of Iranian isolates of BYDV-PAV and CYDV-RPV was found necessary. In fact, there is no sequence information published for BYDV/CYDV from this part of the world, and only a few CYDV-RPV isolates have been cloned and sequenced worldwide (Miller et al., 2002). Therefore, the objective of this study was to determine the partial sequences of Iranian isolates of BYDV-PAV and CYDV-RPV and to study their relationships in comparison to other isolates in the family *Luteoviridae*.

An isolate of BYDV-PAV was obtained from wheat (*Triticum aestivum*) with yellowing symptoms in Shiraz. CYDV-RPV was isolated from a barley plant (*Hordeum vulgare*) with distinct yellowing and dwarfing symptoms in the field in Eidge (Fars province, southwestern Iran). The viruses were identified by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as previously described (Henry and Francki, 1992) using local (Khatabi et al., 2004a; Rastgou et al., 2004a) or commercial (Bioreba, Switzerland) polyclonal antibodies.

BYDV-PAV was transferred and propagated on oat (*Avena sativa* cv. Coast Black) using *R. padi*. CYDV-RPV was maintained by repeated aphid transfer on *A. sativa* cv. Clintland 64 with *R. padi* nymphs. Aphids were fed on the infected plants for a 2-day acquisition feeding and then transferred to healthy oat seedlings at 1–2 leaf stage. After an inoculation access period of 5 days, the aphids were killed with an insecticide spray. The inoculated plants were kept at 18 °C in a growth chamber (Halbert et al., 1992), and after 10 days were tested by DAS-ELISA. The infected tissues were collected four weeks after inoculation, ground to a fine powder in liquid nitrogen and kept at −60 °C. BYDV-PAV and CYDV-RPV were purified as described by Habili et al. (1987) with some alterations (Khatabi et al., 2004a; Rastgou et al., 2004a). Genomic RNA was isolated from purified virus as previously described (Robertson et al., 1991).

The central part of the CP gene (533 nt) from BYDV-PAV and CYDV-RPV can be amplified by reverse-transcription polymerase chain reaction (RT-PCR) using the primer pair Lu1/Lu4, which is general for luteoviruses (Robertson et al., 1991). The sequences of specific primers for BYDV-PAV (BYDV-1: 5′-GTTCTGCCTCAACATCGGAT-3′ and BYDV-2: 5′-AATAGGTAGACTCCTCAAC A-3′) and CYDV-RPV (CYDV-1: 5′-GTCCTTA GATCCAATGGCAAT-3′ and CYDV-2: 5′-CAG CTATCTGAAACCAGTAGA-3′) were kindly provided by Professor W.A. Miller (Iowa State University, USA). RT and PCR were performed subsequently in two steps. In the RT reaction, 2 µl of purified nucleic acids (0.2 µg), 2 µl of specific or degenerate reverse primers (10 mM) and 8.5 µl DEPC-treated water were incubated at 85 °C for 5 min. The mixture was chilled immediately for 5 min on ice and 7 µl of the solution was used as a template for RT. The RT mixture contained 5 µl 5 × RT buffer (100 mM Tris–HCl, 50 mM Triton X-100, 50 mM $MgCl_2$), 2.5 µl dNTPs (10 mM), 1 µl of MLMV reverse transcriptase (25 u μl^{-1}, Roche, Germany), 0.5 µl RNasin (50 u μl^{-1}, Roche) and 9 µl of DEPC-treated water. The RT mixture was incubated for 1 h at 42 °C. PCR was run in a total volume of 25 µl containing 3 µl 10 × PCR buffer, 2 µl $MgCl_2$ (50 mM), 2 µl dNTPs (10 mM), 0.2 µl Taq DNA polymerase (10 u μl^{-1}, Roche) and 2 µl RT reaction. PCR with primers Lu1 and Lu4 were subjected to 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 45 °C, and extension for 1 min at 68 °C, as well as a final extension at 68 °C for 10 min. The thermal cycling scheme for reac-

tions with primers specific for BYDV-PAV or CYDV-RPV was as follows: 2 min at 94 °C, 40 cycles of 30 s at 94 °C, 45 s at 60 °C and 1 min at 72 °C, and a final extension at 72 °C for 10 min. Amplification using specific primers for the CP gene of BYDV-PAV (primers BYDV-1/BYDV-2) and CYDV-RPV (primers CYDV-1/CYDV-2) resulted in the expected sizes of 744 and 719 bp, respectively. Using the degenerate primers Lu1 and Lu4, a product of 1264 bp was obtained for PAV-IR, while the expected size was 533 bp. This was probably caused by mis-priming during RT or PCR.

Amplified products were purified via the PCR DNA Purification Kit (Roche) according to the instructions of the manufacturer. The PCR products were inserted into a pTz57R/T vector using Ins T/A clone PCR product kit (MBI, Fermentas, Vilnius, Lithuania). Recombinant plasmids were used to transform DH5α competent cells of *Escherichia coli*, and were subsequently purified using High Pure Plasmid Kit (Roche). Two clones of each isolate were sequenced from both directions. All sequencing reactions were carried out at Macrogen Inc. facilities (Seoul, Korea) using an ABI 3700 DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA).

Sequence identities were verified by a BLAST search of the GenBank nucleotide database (http://www.ncbi.nlm.nih.gov/blast). Nucleotide and predicted amino acid sequences were aligned, analysed and compared with those of other virus isolates from the family *Luteoviridae* that were available in GenBank (Table 1) using CLUSTAL W (Thompson et al., 1994). Phylogenetic analyses were carried out using Phylogenetic Analysis Using Parsimony (PAUP), version 4 (Swofford, 2002). The distance matrices for the neighbour-joining analyses were calculated using the Kimura two-parameter model. The results obtained from the neighbour-joining analyses were further assessed by parsimony analysis. Essentially the same topologies were obtained with both methods. The robustness of the internal branches of the trees was estimated by bootstrap analysis using 1000 replications.

Sequencing of the cloned RT-PCR products revealed that the longer fragment of PAV-IR contained parts of the CP gene (ORF3; nt 1–526), the MP gene (ORF4; nt 1–428), and the RTP gene (ORF3 + ORF5; nt 1–1264). PAV-IR showed the highest nucleotide identity (97%) to PAV-P from USA (Ueng et al., 1992) and PAV-JPN from Japan (D85783). A high identity (96%) was also found with PAV-4 and PAV-b from France (Papura et al., 2002), as well as with PAV-ILL from USA (AF235167). The amplification product of RPV-IR consisted of parts of the CP gene (nt 1–495), the MP gene (nt 1–366), and the RTP gene

Table 1. Name, origin and description of isolates used in this study

Virus isolates	Abbreviation	Accession number
Determined in this study		
Barley yellow dwarf virus-PAV-Iran	PAV-IR	AY450454
Cereal yellow dwarf virus-RPV-Iran	RPV-IR	AY450425
From database		
Barley yellow dwarf virus-PAV-P	PAV-P	D11032
Barley yellow dwarf virus-PAV-JPN	PAV-JPN	D85783
Barley yellow dwarf virus-PAV-ILL	PAV-ILL	AF235167
Barley yellow dwarf virus-PAV-13	PAV-13	AY040343
Barley yellow dwarf virus-PAV-b	PAV-b	AY040344
Barley yellow dwarf virus-PAV-4	PAV-4	AF391101
Barley yellow dwarf virus-PAV-FHv2	PAV-FHv2	AJ007492
Barley yellow dwarf virus-PAV-FHv1	PAV-FHv1	AJ007491
Barley yellow dwarf virus-PAV-Aus	PAV-Aus	X07653
Barley yellow dwarf virus-PAS-129	PAS-129	AF218798
Barley yellow dwarf virus-MAV-PS1	MAV-PS1	D11028
Cereal yellow dwarf virus-GPV	GPV	AF216863
Cereal yellow dwarf virus-RPS	RPS	AF235168
Cereal yellow dwarf virus-RPV-Aus	RPV-Aus	AF020090
Cereal yellow dwarf virus-RPV-NY	RPV-NY	D10206
Potato leafroll virus-Pol	PLRV-Pol	X74789

(nt 1–719). RPV-IR had the highest nucleotide identity (91%) to RPV-NY from USA (Vincent et al., 1991), but showed also a similar level of identity (90%) to RPV-Aus from Australia (Wang et al., 1998) and RPS from Mexico (Miller et al., 2002). Substitutions in the nucleotide sequences were distributed randomly throughout ORFs 3, 4, and 5 for PAV-IR and RPV-IR. No insertions or deletions were found in the nucleotide sequences of the two Iranian isolates.

A phylogenetic analysis using the CP amino acid sequences of PAV-IR and RPV-IR, and 25 other isolates from the family *Luteoviridae* showed two main well-supported clades (bootstrap value 100) correlating with the genera *Luteovirus* and *Polerovirus* (not shown). PAV-IR grouped among the other PAV isolates in the luteovirus clade (bootstrap value 100), while RPV-IR was located in the polerovirus clade together with CYDV-RPV, CYDV-RPS and CYDV-GPV (bootstrap value 100). A phylogenetic analysis of 1264 nt from the CP/RTP region of PAV, MAV, and PAS (Figure 1a) confirmed the close relationships between PAV-IR, PAV-P, PAV-ILL and PAV-JPN (bootstrap value 100). In the phylogenetic analysis of 719 nt from the CP/RTP region of PLRV, GPV, RPV and RPS (Figure 1b), RPV-IR grouped with the RPV and RPS isolates (bootstrap value 100).

A recent study of the diversity of BYDV-PAV showed that the isolates of this species could be divided into two groups (I and II) based on the CP nucleotide sequence (Bisnieks et al., 2004). PAV-IR was closely related with PAV group I isolates from

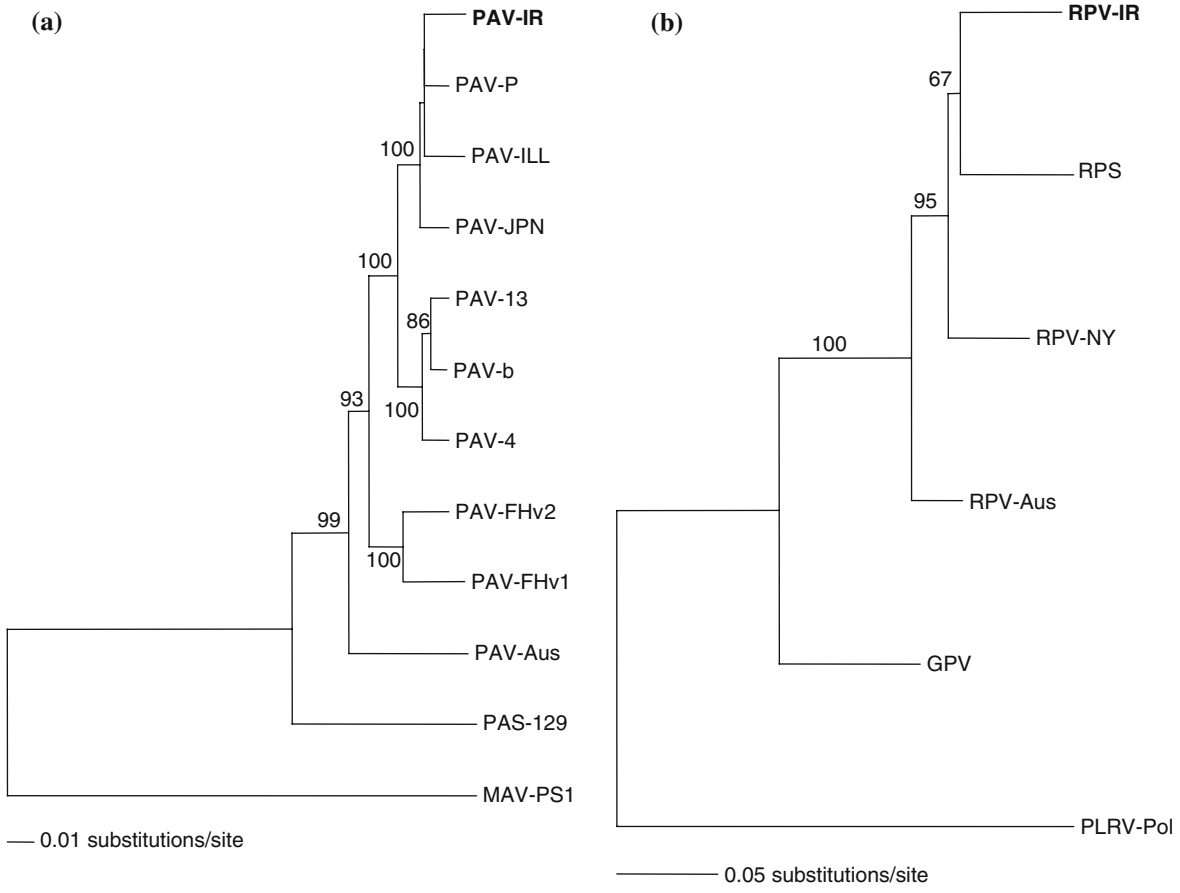

Figure 1. Neighbour-joining analyses showing predicted relationships between isolates from the family *Luteoviridae* based on the alignment of 1264 nt (genus *Luteovirus*) or 719 nt (genus *Polerovirus*) of the CP, MP and RTP genes. The Iranian isolates determined in this study are indicated in bold. Numbers represent the bootstrap values out of 1000 replicates. Only bootstrap values higher than 50 are shown. Horizontal lines are in proportion to the number of nucleotide differences between branch nodes. (a) Relationships of PAV-IR to isolates from the genus *Luteovirus*. (b) Relationships of RPV-IR to isolates of the genus *Polerovirus*.

other regions of the world. The result shows that this genotype of PAV is present also in Iran. While there was no geographic grouping found for PAV, a correlation with host species has been suggested (Bisnieks et al., 2004). PAV isolates from oat and barley have been placed into group I, while PAV isolates from grasses and barley have been placed in group II. PAV-IR was initially isolated from wheat and then maintained on oat. PAV-IR is to our knowledge the first PAV isolate characterised from wheat. A larger number of virus isolates from different hosts needs to be sequenced to verify the suggested grouping according to host species.

CYDV-RPV has a worldwide distribution (Lapierre et al., 2004), but so far there is sequence information from only two isolates: RPV-NY from USA (Vincent et al., 1991) and RPV-Aus from Australia (Wang et al., 1998). CYDV-RPS has been found only in Mexico and California (Henry et al., 2004), and there is one sequence available from a Mexican isolate (Miller et al., 2002). The sequence identity between RPV and RPS is high in the CP region, but the amino acid sequence identity for ORFs 0, 1 and 2 is only 41%, 55% and 81%, respectively (Miller et al., 2002). The sequence information confirmed the identification of an RPV-like virus in Iran. However, RPV-IR showed a similar sequence identity with both RPV and RPS (90–91%), and the phylogenetic analyses suggested that it may be more closely related to RPS. More sequencing, especially of the 5′-end of the genome, is required to further clarify the identity of this virus.

This paper reports the first molecular identification of BYD-causing viruses in Iran and southwest Asia. Close relationships were found with isolates of BYDV-PAV and CYDV-RPV/RPS from other parts of the world. To obtain a more complete picture of the diversity among isolates of BYDV-PAV and CYDV-RPV in the region, more isolates should be sequenced and studied from different geographic locations and hosts, including both cultivated and non-cultivated plants.

Acknowledgements

We wish to thank Prof. W. A. Miller for designing primers for BYDV-PAV and CYDV-RPV, for seeds of *A. sativa* cv. Clintland 64 and his helpful comments. We are also grateful to Dr. M. Henry for sending positive controls, seeds of *A. sativa* cv. Coast Black and her helpful papers and comments. We also want to thank Dr. Afsharifar and Mr. Masoumi for their helpful comments.

References

Bisnieks M, Kvarnheden A, Sigvald R and Valkonen JPT (2004) Molecular diversity of the coat protein-encoding region of *Barley yellow dwarf virus-PAV* and *Barley yellow dwarf virus-MAV* from Latvia and Sweden. Archives of Virology 149: 843–853

Chay CA, Gunasinge UB, Dinesh-Kumar SP, Miller WA and Gray SM (1996) Aphid transmission and systemic plant infection determinants of barley yellow dwarf Luteovirus-PAV are contained in the coat protein read through domain and 17-kDa protein, respectively. Virology 219: 57–65

D'Arcy CJ and Burnett PA (1995) Barley yellow dwarf virus: A brief introduction. In: D'Arcy CJ and Burnett PA (eds) Barley Yellow Dwarf: 40 Years of Progress (pp. 1–5) APS Press, St. Paul, Minnesota, USA

D'Arcy CJ, Domier LL and Mayo MA (2000) Family *Luteoviridae*. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR and Wickner RB (eds) Virus Taxonomy: Seventh Report of the International Committee on the Taxonomy of Viruses (pp. 775–784) Academic Press, San Diego, California, USA

D'Arcy CJ, Domier LL and Torrance L (1999) Detection and diagnosis of luteoviruses. In: Smith HG and Barker H (eds) The Luteoviridae (pp. 147–168) CAB International Publishing, Oxford, UK

Habili N, McInnes JL and Symons RH (1987) Nonradioactive, photobiotin-labeled DNA probes for the routine diagnosis of barley yellow dwarf virus. Journal of Virological Methods 16: 225–237

Halbert SE, Connelly BJ, Lister R, Klein RE and Bishop GW (1992) Vector specificity of barley yellow dwarf virus serotypes and variants in southwestern Idaho. Annals of Applied Biology 121: 123–132

Henry M, Beckett R and Miller WA (2004) Cereal yellow dwarf associated to CYDV-RPS. In: Lapierre H and Signoret P-A (eds) Viruses and Virus Diseases of Poaceae (Graminae) (pp. 561–562) INRA Editions, Paris, France.

Henry M and Francki RIB (1992) Improved ELISA for the detection of barley yellow dwarf virus in grasses. Journal of Virological Methods 36: 231–238

Khatabi B, Rastgou M and Izadpanah K (2004a) Purification and antiserum preparation of Iranian isolate of cereal yellow dwarf virus-RPV. Proc.16th Iranian Plant Protection Congress, 82

Khatabi B, Rastgou M, Masoumi M, Afsharifar AR and Izadpanah K (2004b) Efficiency of cereal aphids in BYDV-MAV and CYDV-RPV transmission. Proc.16th Iranian Plant ProtectionCongress, 80

Lapierre H, Henry M, Beckett R and Miller AW (2004) Cereal yellow dwarf associated with CYDV-RPV. In: Lapierre H

and Signoret P-A (eds) Viruses and Virus Diseases of Poaceae (Graminae) (pp. 563–565) INRA Editions, Paris, France

Mayo MA (2002) ICTV at the Paris ICV: Results of the plenary session and the binomial ballot. Archives of Virology 147: 2254–2260

Miller WA, Beckett R and Liu S (2002) Structure, function and variation of barley yellow dwarf virus genomes. In: Henry M and MacNab A (eds) Barley Yellow Dwarf Diseases Recent Advances and Future Strategies (pp. 1–8) CIMMYT, Mexico

Miller WA, Waterhouse PM, Kortt AA and Gerlach WL (1988) Sequence and identification of the barley yellow dwarf virus coat protein gene. Virology 165: 306–309

Papura D, Jacquot E, Dedryver CA, Luche S, Riault G, Bossis M and Rabilloud T (2002) Two-dimensional electrophoresis of proteins discriminates aphid clones of *Sitobion avenae* differing in BYDV-PAV transmission. Archives of Virology 147: 1881–1898

Rastgou M, Khatabi B and Izadpanah K (2004a) Purification and antiserum preparation of Iranian isolate of barley yellow dwarf virus-MAV. Proc.16th Iranian Plant Protection Congress, 64

Rastgou M, Khatabi B, Yasaei A, Afsharifar AR, Masoumi M and Izadpanah K (2004b) Distribution and new hosts of viruses causing yellow dwarf in cereals. Proc. 16th Iranian Plant Protection Congress. 61

Robertson NL, French R and Gray SM (1991) Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. Journal of General Virology 72: 1473–1477

Swofford DL (2002) PAUP. Phylogenetic analysis using parsimony (and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA

Thompson JD, Higgins DG and Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680

Ueng PP, Vincent JR, Kawata EE, Lei CH, Lister RM and Larkins BA (1992) Nucleotide sequence analysis of the genomes of the MAV-PS1 and P-PAV isolates of barley yellow dwarf virus. Journal of General Virology 73: 487–492

Vincent JR, Lister RM and Larkins BA (1991) Nucleotide sequence analysis and genomic organization of the NY-RPV isolate of barley yellow dwarf virus. Journal of General Virology 72: 2347–2355

Wang M-B, Cheng Z, Keese P, Graham MW, Larkin PJ and Waterhouse PM (1998) Comparison of the coat protein, movement protein and RNA polymerase gene sequences of Australian, Chinese, and American isolates of barley yellow dwarf virus transmitted by *Rhopalosiphum padi*. Archives of Virology 143: 1005–1013