Acta virologica **57**: 383 – 385, 2013 doi:10.4149/av_2013_03_383

LETTER TO THE EDITOR

Coat protein based molecular characterization of *Barley yellow dwarf virus* isolates identified on oat plants in Pakistan

M. ALI[1], M. TAHIR[1*], S. HAMEED[2], M. ASHRAF[1]

[1]Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Sector H-12, Islamabad; [2]Crop Diseases Research Institute (CDRI), National Agricultural Research Center (NARC), Park Road, Islamabad

Received May 7, 2013; accepted July 23, 2013

Summary. – *Barley yellow dwarf virus* (BYDV) is a potential threat to the agriculture production. The amplified complete coat protein sequences of the isolate M07 and M12 were determined to be 597 bp and 603 bp, respectively. M07 showed maximum nucleotide sequence identity of 87.6% (84.3% amino acid sequence identity) to a Chinese isolate of BYDV-PAV. Whereas, the isolate M12 showed maximum nucleotide sequence identity of 94.5% (94.0% amino acid sequence identity) to French isolate BYDV-PAV. Since more than 10 % differences, among the amino acid level of any gene product, is the sole criterion to discriminate between species within the family *Luteoviridae*, the isolate M07 that shows maximum of 84.3% (less than 90%) amino acid sequence identity with previously known *Luteovirus* species, is thus, recommended to be a distinct PAV species within the genus *Luteovirus*.

Keywords: Luteovirus; oat; reverse transcription

Oat (*Avena sativa* L.; *Poaceae*) is an important fodder (spring season; multi-cut) crop grown throughout Pakistan both under irrigated and rain-fed conditions. Symptoms specific to *Barley yellow dwarf virus* (BYDV) were first witnessed in Pakistan, in 1964 (*1*). Oat leaf samples showing leaf reddening (Fig. 1), symptoms typical of BYDV, were collected from areas around Islamabad (isolate M07) and Peshawar (isolate M12) in Pakistan, during the year 2011. Total RNA was extracted and cDNA was synthesized using specific primer pair CP+ (*2*) and Lu4 (*3*). The expected size amplicons (approx. 600 bp) were obtained and cloned into T/A vector – pTZ57R/T (Thermo Scientific). The potential clones were sequenced in both orientations. Sequences were assembled and analyzed using Lasergene package (DNA Star Inc. Madison, WI, USA). Multiple sequence alignment and phylogenetic tree was constructed using ClustalX (*4*). The tree was displayed, manipulated and printed using Treeview (*5*).

The complete coat protein sequence, from isolate M07 [EMBL:HE584721] and M12 [EMBL:HE584722], was determined to be 597 bp and 603 bp long, respectively. Sequence comparison revealed two amino acid deletions at position 14 and 15 in the isolate M07. The two isolates shared 79.2% nucleotide sequence identity (71.2% amino acid sequence identity) between each other (Table 1). The coat protein sequence derived from the isolate M07 showed maximum nucleotide sequence identity of 87.6% (84.3% amino acid sequence identity) (Table 1) to a Chinese isolate [GenBank: EU332315] of BYDV-PAV. Whereas, M12 showed 94.5% nucleotide sequence identity (94.0% amino acid sequence identity) (Table 1) to a French isolate [GenBank: AY167108] of BYDV-PAV.

To sum up, the isolate M07 and M12 showed 84.3% and 94.0% maximum amino acid sequence identity to previously known BYDV-PAV isolates (Table 1). Since, the present

*Corresponding author. E-mail: gullsbs@yahoo.com; phone: +92-5-90856131.

Abbreviations: BYDV = Barley yellow dwarf virus

Fig. 1

Oat (*Avena sativa*) plants showing reddening of the leaves – symptomatic to BYDV infection

Table 1. Highest and lowest percent nucleotide (nt) sequence identities and amino acid (aa) sequence identities (in parenthesis) of M07 and M12 with other selected BYDV and CYDV isolates available in the database

Virus No. of isolates	RPV nt (aa)	GPV nt (aa)	MAV nt (aa)	PAV-III nt (aa)	PAV-II nt (aa)	PAV-I nt (aa)	M12 nt (aa)	M07 nt (aa)
M07 [01]	36.7-37.2 (37.8-39.4)	39.0-39.7 (39.4-40.4)	73.9-74.0 (65.1-65.6)	76.0-78.4 (68.7-71.2)	75.5-79.2 (66.1-71.2)	85.1-87.6 (82.8-84.3)	79.2 (71.2)	** (**)
M12 [01]	42.4-43.8 (43.5-44.5)	43.9 (43.0-43.5)	73.2 (69.8)	88.4-89.2 (85.4-86.6)	93.0-94.5 (90.6-94.5)	78.2-80.3 (72.4-73.9)	** (**)	
PAV-I [08]	38.7-43.2 (37.7-42.2)	39.7-42.3 (37.7-39.7)	70.3-73.0 (63.8-67.3)	75.0-80.2 (70.8-73.9)	75.3-82.2 (68.2-73.9)	90.7-99.0 (91.9-100)		
PAV-II [12]	40.3-43.9 (43.0-46.2)	41.1-46.9 (41.0-45.2)	73.2-76.3 (67.8-72.0)	88.1-90.1 (83.3-87.0)	92.3-99.8 (90.1-99.5)			
PAV-III [09]	41.6-43.7 (43.0-46.9)	43.9-46.0 (42.5-45.8)	73.0-74.8 (68.3-69.3)	97.0-99.8 (95.8-100)				
MAV [02]	37.8-40.7 (40.7-41.2)	40.0-40.7 (37.7-38.7)	99.7 (99.5)					
GPV [02]	77.6-78.2 (70.1-71.6)	99.2 (99.0)						
RPV [02]	95.0 (94.1)							

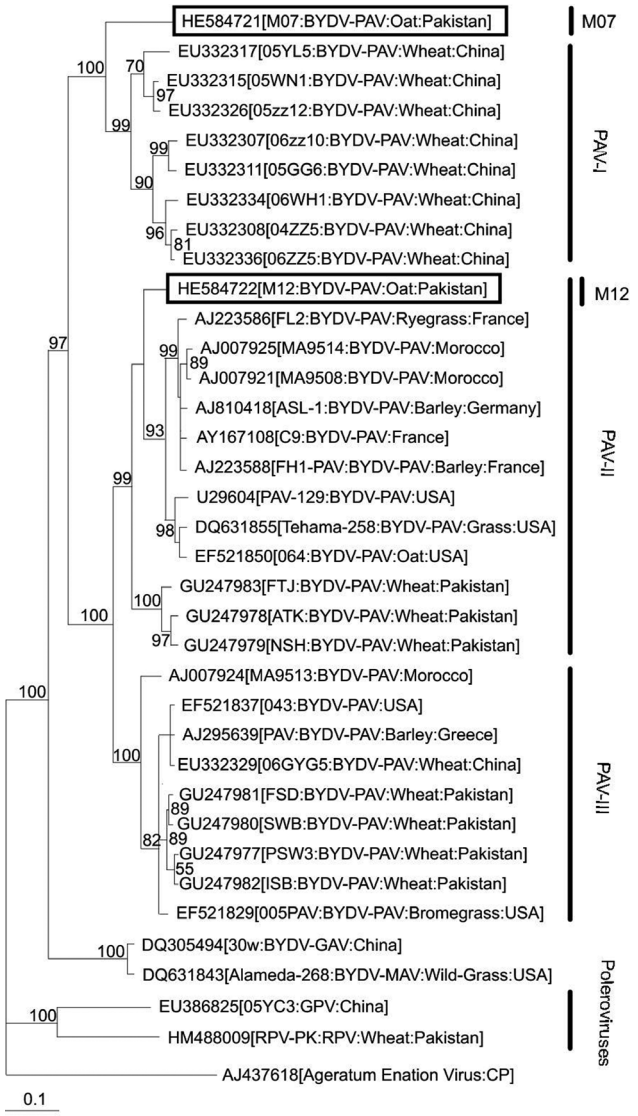

Fig. 2

Phylogenetic tree based upon alignments of the complete nucleotide sequences of the ORF3 (CP) of BYDV identified here with selected sequences available in the databases

ICTV criterion based on > 10 % amino acid differences in any gene product discriminate between species within the family *Luteoviridae* (*6*), the isolate M07 may be considered as a distinct species. A phylogenetic tree, based on complete coat protein gene sequences also showed that the isolate M07 occupies a distinct position, which is supported by high (100%) bootstrap confidence values. However, the isolate M12, as expected, was clustered within BYDV PAV-II (Fig. 2).

Robertson and French (*7*) avoided inclusion of some BYDV isolates, into a particular *Luteovirus* species (i.e. MAV) based on the criterion of 90% amino acid identity of CP region, and thus regarded them as Alaska MAV-like species. The isolate M07, identified here, showing highest level of 84.3% amino acid sequence identity to any previously characterized serotype of BYDV, may be considered as distinct BYDV-PAV species within the genus *Luteovirus*, as well.

References

1. Aslam M, Ahmad I, In Proc. World perspectives on Barley yellow dwarf. July, 6–11. Udine, Italy. p. 85, 1987.

2. Liu F, Wang X, Liu Y, Xie J, Gray SM, Zhou G, Gao, Arch. Virol., 152, 1365–1373, 2007. http://dx.doi.org/10.1007/s00705-007-0947-8

3. Robertson NL, French R, Gray SM, J. Gen. Virol. 72, 1473–1477, 1991. http://dx.doi.org/10.1099/0022-1317-72-6-1473

4. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, Nucleic Acids Res. 25, 4876–4882, 1997. http://dx.doi.org/10.1093/nar/25.24.4876

5. Page RDM, Comp. App. Biosci., 12:357–358, 1996.

6. D´Arcy CJ, Domier LL, In: Fauquet CM, Mayo MA, Mannilof J, Desselberger U, Ball LA (Eds): Virus Taxonomy. VIIIth Report of ICTV. London, Elsvier/Academic Press, pp. 891–900, 2005.

7. Robertson NL, French RC, Arch. Virol. 152, 369–382, 2007. http://dx.doi.org/10.1007/s00705-006-0846-4