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Coat protein gene based phylogenetic analysis of barley yellow dwarf virus-PAV infecting cereal

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Abstract

Barley Yellow Dwarf disease exclusively afflicts plant species in the family *Poaceae* and is especially noticeable where ever crops such as barley, maize, oats and wheat are cultivated. The total RNA extracted through TRI reagent and Reverse transcription Polymerase chain reaction (RT-PCR) was carried out by using Revert Aid^{TM} H-minus. For confirmation, Coat Protein Gene (CpG) specific primers of BYDV-PAV were used. The result showed that BYDV-PAV exhibit a high frequency of nucleotide and amino acid homogeneity within CpG region. As compared with inter population the isolate JQ811488 (from oat) found a unique thread of 3 amino acid difference at 3` "ANP" while isolate JQ811487 (from wheat) showed 9aa differences mostly at 5`. Pakistani isolate JQ811489 (from maize) found maximum 99.2% similarity with US isolate DQ285673 which is highest as compare with inter population. This study will increase understanding of the genetic diversity of Pakistani isolates of BYDV and their relationship among and with other isolates.

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Introduction

Barley Yellow Dwarf Virus (BYDV) infects a wide range of Poaceae family including major cereal crops like wheat, barley oats and maize (Lister and Ranieri 1995). Detection methods are also important for perceptive virus biology and relationships with different hosts (Gibbs *et al.*, 2008). BYDVs are transmitted by aphids having 5.5 to 5.8kb genome and ORF3 encodes the major coat protein gene while translates from the sub genomic RNA1 (Miller *et al.*, 2002). The nucleic acid of two different BYDV viruses analyzed with two different vectors species and derived the RTD involves in vector specificity or vector determination of viruses (Brault *et al.* 2007). ORF 3 encodes the 22-kDa coat protein which embedded ORF 4 (Gray and Gildow, 2003) translated by leaky scanning, encodes a 17-kDa protein that cause systemic infection in cereal crops (Elena *et al.*, 2008). The coat protein (Cp) gene was genetically divergent in PAS species but not in species PAV. This is due to genetic float, since there was no evidence that deviate any neutral model of gene evolution under positive selection (Hall, 2006).

Viruses are often considered as the most damaging parasitic agents, but this view is strongly biased towards economic impacts (Li *et al.*, 2001). Early studies suggested that some plant RNA viruses evolved more slowly than RNA viruses that infect animals. Mostly evolutionary research has been focused on RNA plant viruses with special emphasis on *Luteoviruses* particularly BYDV (Pagán and Holmes 2010). The present study was undertaken to determine the genetic diversity of Pakistani isolates of BYDV and their relationship among and with other known isolates.

Materials and methods

Sample collection

The BYDV-PAV positive samples from cereal crops; wheat, oat and maize, National Agriculture Research Center, Islamabad and analyzed on the basis of high optical density value through Enzyme Linked Immuno Sorbent Assay (ELISA). After confirmation of BYDV-PAV through ELISA, The samples were further processes for molecular diversity on the basis of coat protein gene.

Total RNA isolation and primers

Leaf tissues were triturated in liquid nitrogen and homogenized in TRI Reagent 118 (1ml:1mg v:m) and incubated at room temperature for 10min. Cold chloroform (0.2ml) was added to the mixture for separation of aqueous phase from organic phase. The aqueous phase was transferred to a fresh centrifuge tube (1.5ml), added 0.5ml Isopropanol and centrifuged at 12,000rpm for 15min. The organic phase was discarded and the RNA pellet was washed with 75% Ethanol and then air dried for 10min. Total RNA was dissolved in nuclease free/DEPC treated water. All centrifugation was done at 4°C.

cDNA synthesis and PCR amplification

Reverse transcription (RT) was carried out on freshly extracted total RNA and reverse primer (*ctatttggccgtcatcaaac*) (@10pMol) using Revert Aid™ H-minus First Strand cDNA Synthesis kit Ferments. RT mixture was incubated at 42°C/60min followed by transcriptase inactivation by heating at 72°C for 7min.

In PCR reaction mixture, components like cDNA template, 10x Taq reaction buffer, $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), Taq DNA polymerase and BPCF3; *"atgaattcagtaggccgtaga"*, BPCR2; *"ctatttggccgtcatcaaac"* primers were used. PCR conditions were standardized by changing denatrization, annealing, extension temperature and time along with cycles were altered to find out the best condition in PCR amplification. Amplified PCR products were analyzed by electrophoresis on a 1 % (w/v) agarose gel in Tris Borate EDTA buffer stained with Ethidium bromide. The results were visualized under UV Transelluminator and product size was determined by using the DNA ladder (molecular weight marker).

Coat protein gene identification of BYDV-PAV

The high OD405 value samples were used for total RNA extraction. Synthesized cDNA through *Revert Aid reverse transcriptase* enzyme for processing of PCR amplification.

The Dream *Taq* DNA Polymerase enzyme amplified coat protein gene. The product size was approximately 600bp when visualized on gel.

PCR purification and direct sequencing

The amplified CpG of BYDV was purified according to manufactured protocol of *Gene JET™ PCR Purification Kit. Binding buffer was added and PCR mixture was transferred to the Gene JET™* purification column. Centrifugation was done and wash buffer was added onto the column, centrifuged and elution buffer was added and again centrifuged for 1 min and the PCR purified products were sequenced from Macrogen Ibc (Korea). The sequences were confirmed by sequencing in forward as well as reverse direction.

Phylogenetic analysis

Sequence determination:The sense and antisense sequences were confirmed through Basic Local Alignment Search Tool (BLAST).

The sequences were further aligned and translated to amino acid sequence. The data transferred to Sequence data explorer for nucleotide and amino acid difference estimation. The differentiation among inter population was find out by 1:2 of difference and similarity respectively through MEGA 6 software.

Phylogenetic tree: The 11 isolates sequences (Table 1) were used to construct a phylogenetic tree through MEGA 6 (Tamura *et al.*, 2013). The relationships of isolates are presented in a treelike form which show the mechanisms of evolutionary history.

All ML phylogenetic analysis were carried out using an initial distance-based starting tree (BIONJ), applying selected substitution models, parameters and with gaps being treated as missing data. Nodes support was assessed using bootstrap analysis with 1000 replicates to estimates the significance of the generated tree.

Table 1. Description of isolates used in phylogenetic analysis.

Isolate Name	Host Name	Origin	Accession No
PAV-PK	Wheat	Pakistan	JQ811487
PAV-PK1	Oat	Pakistan	JQ811488
PAV-PK2	Maize	Pakistan	JQ811489
BYDV FL3-PAV	Ryegrass	France	AJ223587
PAV C9	Cereals	France	AY167108
PAV WA95	Maize	USA	DQ285673
PAV WG13	Wheat	USA	DQ285675
Y108	Wheat	China	FJ875303
CO374_79	Maize	Brazil	JX067842
CO373_94	Maize	Brazil	JX067851
TR	Wheat	Turkey	KC900900

Results and discussion

BYDV-PAV sequences

The coat protein gene of BYDV-PAV was found to be 603 nucleotides long, encoded 201 amino acids. Gen Bank assigned accession number to wheat isolate as JQ811487, oat as JQ811488 and to maize isolate as JQ811489. The nucleotide translated DAG sequence is underlined (Fig. 1) and the yellow color is showing amino acid differences. The inter isolate sequences showed 50 nucleotide and 18 amino acid differences. The isolate JQ811487 showed 9aa differences mostly found at 5` "KSTQPLSGN".

The isolate JQ811488 showed 4aa differences mostly found at 3` "AANP". The isolate JQ811489 showed only 2aa differences found on 5` "LK".

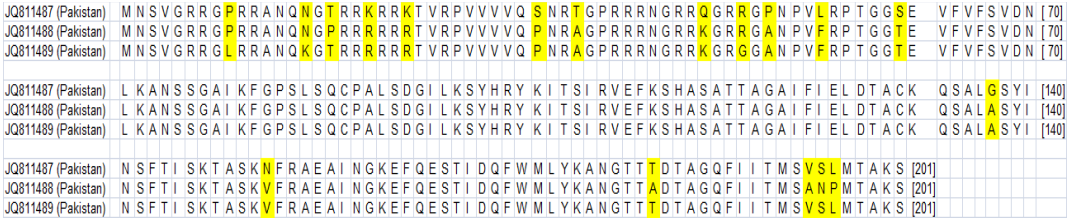

Fig. 1. Inter population (JQ811487, JQ811488 and JQ811489) amino acids sequence of BYDV CpG.

Phylogenetic analysis of Cp gene from different hosts of BYDV-PAV

The total 11 coat protein gene nucleotide sequences were used to construct this phylogenetic tree Fig. 2, where 3 isolates were from Pakistan and 8 isolates from different regions of the world i.e. China, France, USA, Brazil and Turkey. The phylogenetic tree showed one major cluster which includes two US isolates DQ285673 and DQ285675, a French isolate AJ223587, a Pakistani isolate JQ811489 and two Brazilian isolates JX067842 and JX067851. The Pakistani isolate JQ811487 and JQ811488 exist in outer clades. The Chinese isolate FJ875303 fall in out group.

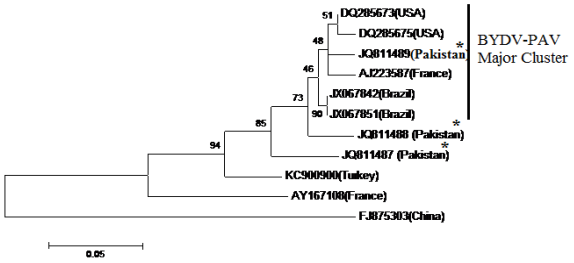

Fig. 2. The Cp gene Phylogenetic tree constructed by using the Maximum Likelihood method on MEGA6.

The percentage similarity index table generated from the pair wise alignment distances (Table 2). The isolate JQ811487 showed maximum 96.6% similarity with US isolate DQ285673 and minimum 84.1% with Chinese isolate FJ875303. The isolate JQ811488 showed maximum 97.8% similarity with Brazilian isolate JX067851 and minimum 83.7% with Chinese isolate FJ875303. The isolate JQ811489 showed maximum 99.2% similarity with US isolate DQ285673 and minimum 82.9% with Chinese isolate FJ875303.

The inter population similarity index of three Pakistani isolates; The isolate JQ811487 (from wheat) showed 96.4% with JQ811488 (from oat) and 96.5% with JQ811487 while the isolate JQ811488 showed 96.9% with JQ811489 (from maize).

In structural protein the Cp gene found highly conserved region in BYDV-PAV (Gray and Gildow 2003). The study found that the Ker isolates have very low diversity 0.6% with other BYDV-PAV isolates (Svanella-Dumas *et al.* 2013).

Table 2. % age similarity index matrix derived from pair wise distances from MEGA6.

Isolates	JQ811487	JQ811488	JQ811489	AJ223587	AY167108	DQ285673	DQ285675	FJ875303	JX067842	JX067851	KC900900
JQ811487 (Pakistan)	100										
JQ811488 (Pakistan)	96.4	100									
JQ811489 (Pakistan)	96.5	96.9	100								
AJ223587 (France)	95.8	97.2	96.9	100							
AY167108 (France)	92.3	91.5	91.6	91.1	100						
DQ285673 (USA)	96.6	96.8	99.2	97.5	92.1	100					
DQ285675 (USA)	96.0	96.6	98.8	97.2	91.5	99.4	100				
FJ875303 (China)	84.1	83.7	82.9	83.1	84.6	83.1	83.3	100			
JX067842 (Brazil)	96.0	97.5	96.9	98.8	92.1	97.3	96.9	84.0	100		
JX067851 (Brazil)	96.2	97.8	97.0	98.7	92.1	97.4	97.0	83.7	99.8	100	
KC900900 (Turkey)	95.6	94.8	94.2	94.3	93.1	95.2	94.6	84.3	94.5	94.8	100

The diverse recombinant pattern in BYDV-PAV and MAV genome found because both transmitted by same aphid vector (Saleem *et al.* 2013). Among BYDV comparison of CP sequences found minimum 60% similarity between the three *Luteoviridae* genera while less than 40% was from *Enamovirus* genus (Mayo and d'Arcy 1999).

The study found that only the geographical base cannot play a role in genetic evolution, because the involvement of host plants is also important in the genetic diversity of BYDV-PAV (Mastari *et al.* 1998). So, the regular molecular identification is essential to observe genetic variation and adaptation of viral genome in nature (Stern and Sorek 2011).

Conclusion

The maximum nucleotide and amino acid similarity among CpG is due to highly conserved region in genome of BYDV-PAV. This basic research will play a vital role for understanding the relationships between virus-vector, origin and virus-host to developing control strategy for BYDV disease infecting cereal crops in Pakistan.

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