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PHYLOGENETIC AND GENOME-WIDE PAIRWISE DISTANCE ANALYSIS OF THE GENUS *LUTEOVIRUS*

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Luteovirus is a phytopathogenic monopartite virus of global importance. Re-assortment and recombination by exchanging genome segments play vital role in the virus evolution. Differences in the breadth and specificity of host range, serology and divergence of >10 % amino acids in any gene product (ORF) are the basis of *Luteovirus* species distinction. To confirm taxonomic status, single ORF that may not accommodate / represent occurrence of genetic recombination (may be of polythetic nature), therefore, may not be sufficient. Instead inference from the whole genome is required. We analyzed distribution of a total of 94 available whole genome *Luteovirus* sequences in a consistent classification based on pairwise sequence comparison. The adjustment of the two outlier sequences, being recognized, is also discussed.
Keywords: Luteoviruses, pairwise sequence comparison, sequence demarcation tool, yellow dwarf disease, aphid-vector.

INTRODUCTION

Luteovirus (family: *Luteoviridae*) is a monopartite, insect-borne, phytopathogen having global importance (Eamens *et al.*, 2008). It is a causal agent of yellow dwarf disease of cereals, can also cause disease in some dicots, recognized for the first time, in 1951 (Oswald and Houston, 1953). The virion particle is non-enveloped, icosahedral (T=3), 25-28 nm in diameter (Miller *et al.*, 2002), phloem-limited (Rochow, 1970) and transmitted from plant to plant by aphids obligately, in a circulative, and non-propagative manner (Gray and Gildow, 2003). It encapsidates a single-stranded messenger-sense ribonucleic acid (+ssRNA) genome, varying in size from 5.6 to 6.0 kb (Liu *et al.*, 2012).

Lack of proof-reading during synthesis of RNA progeny contributes towards high mutation frequencies. This makes luteoviruses evolve at a rate of 3.158×10^{-4} nucleotide substitutions/site/year (Worobey and Holmes, 1999), similar to other RNA viruses. RNA recombination has been known to occur between similar as well as distantly related viruses. High frequency of homologous recombination is evident in cereal infecting luteoviruses (Wu *et al.*, 2011) that may explain their evolutionary success. Several recombinant genomes have been reported from luteovirids e.g. *Cucurbit aphid-borne yellows virus* [genus: *Polerovirus*] is a derived recombinant genome of polero-like and enamo-like parental viruses (Gibbs *et al.*, 2000; Gibbs and Cooper, 1995); *Sugarcane yellow leaf virus* [genus: *Polerovirus*] is derived from polero-, luteo- and enamo-like viruses (Chinnaraja *et al.*, 2013; Smith *et al.*, 2000); *Soybean dwarf virus* [genus: *Luteovirus*] is a probable recombinant of luteo-like and polero-like parental viruses (Rathjen *et al.*, 1994; Terauchi *et al.*, 2001) and *Bean leaf roll virus* [genus: *Luteovirus*] is the product two independent recombinant events between luteo-like and polero-like virus ancestors (Domier *et al.*, 2002). Recently, it has been known that exchange of hereditary material may also have occurred between two different BYDV serotypes (Boulila, 2011).

Template-strand switching of RNA dependent RNA polymerase is known to have a role in RNA recombination and is considered as a major driving force in the virus evolution. In luteoviruses, replicase strand-switching at sgRNA promoters has been known (Miller *et al.*, 1997) with sgRNA1 promoter as a putative recombination hotspot (Koev *et al.*, 1999). As a consequence of variance in the selection pressure or RNA recombination, different grouping of 5′ and 3′ halves have been evolved (Miller *et al.*, 1995). BYDV isolates, therefore, cluster into different groups when 5′ half sequences were considered in comparison with their 3′ halves (Chalhoub *et al.*, 1995).

The genetic re-assortment and recombination, although, contributed to the formation of relatively stable viral entities with distinguishable biological properties, create genomes with mosaic / multiple features. The polythetic or cluster class (van Regenmortel *et al.*, 2013), thus, could not be distinguished if one or few ORFs are considered. Whole genome information may distinguish them from other related viruses from which they evolved. The high frequency of recombination occurs may be as a consequence of mixed infection of distinct viruses. As an instance, co-infection of BYDV distinct species, also known as serotypes (PAV and PAS), have been found in a pearl millet leaf sample

Table 1. International committee on taxonomy of viruses recognized species within the genus *Luteovirus*. Corresponding accession numbers of type members are given.

Sr.	Virus Species	Acc. No. (type member)
1	*Barley yellow dwarf virus-MAV* (BYDV-MAV)	D01213
2	*Barley yellow dwarf virus-PAS* (BYDV-PAS)	AF218798
3	*Barley yellow dwarf virus-PAV* (BYDV-PAV)	X07653
4	*Barley yellow dwarf virus-ker*II (BYDV-kerII)	KC57719
5	*Barley yellow dwarf virus-ker*III (BYDV-kerIII)	KC559092
6	*Rose spring dwarf-associated virus* (RSDaV)	EU024678
7	*Bean leafroll virus* (BLRV)	AF441393
8	*Soybean dwarf virus* (SbDV)	L24049

(unpublished data). Co-infections or multiple viral infections may be due to maritime trade, at least, for instance in between USA and Australia (Malmstrom *et al.*, 2007).

***Existing classification of Luteovirus species*:** Based on the breadth and specificity of host range, aphid-vector type and the criterion of >10% amino acid divergence for any gene product (D'Arcy *et al.*, 2005), the isolates within the genus *Luteovirus* have been grouped into several distinct species (Ali *et al.*, 2013; Robertson and French, 2007). So far, eight species have been recognized (ICTV 9th report and Adams *et al.* 2014). Primarily, these *Luteovirus* species (Table 1) have been named according to their first isolation host (Ali *et al.*, 2014) plants (e.g. barley, rose, soybean). Also, the virus species names describe the prevalent induced symptoms (e.g. yellow dwarf and leaf roll).

Conventionally, all cereal infecting luteoviruses were named as Barley yellow dwarf viruses (BYDVs). To discriminate between different species within the virus group, different acronyms were added, such as BYDV-PAV, BYDV-PAS, and BYDV-MAV, based on their prevalent characterized insect-vector aphids (Rochow, 1969). For instance, BYDV-MAV is preferentially transmitted by *Macrosiphon avenae* (later on named as *Sitobean avenae*), thus named as MAV. By concerning nomenclature, it looks difficult to accommodate a virus harbored by two different aphids in the same virus species. Specifically, BYDV-GAV (isolate of MAV) is transmitted equally by two aphid vectors – *Shizaphis graminum* and *Macrosiphon avenae* (Wang *et al.*, 2000). Recently, two new species have been named according to their geographic location (i.e. *kerII* and *kerIII*).

Antigenic features of luteoviral transmission cannot be linked to their aphid vectors (Du *et al.*, 2007). Specifically, polyclonal antibodies cannot distinguish PAV and PAS, although they are regarded as distinct species. Some authors considered their isolates as distinct species based only on CP and/or MP amino acid identity (Ali *et al.*, 2013; Robertson and French, 2007). Previously, BYDV PAV-USA clade has been known to be distinct from PAV-Chinese clade (Boulila, 2011). A full length BYDV Chinese-isolate (PAV-CN) has been recommended as a new species (Liu *et al.*, 2007), based on sequence identities of individual ORFs (>10% amino acid divergence criterion), perceived to be evolved through genetic recombination. Some luteoviruses showed extensive recombination, exchanging parts of their genomes, which make them associated with two or more distinct species based on ORF sequences. However, >10% amino acid divergence rule may not distinguish them as distinct species. They may be members of distinct species, if full genomes are considered for the classification.

MATERIALS AND METHODS

To validate the existing rule of >10% amino acid discrimination, amino acid sequences of complete ORFs of luteoviruses were retrieved from the GenBank. The sequences were aligned using Clustal W (Larkin *et al*., 2007) and/or MUSCLE (Edgar, 2004) algorithms. Percent amino acid identities and phylogenetic trees of the ORF were produced. For full length virus sequence analysis, nucleotide sequences corresponding to complete genome of luteoviruses were retrieved from the GenBank (dated on Dec. 30 2014). MUSCLE algorithm (Edgar, 2004) was used to align the sequences, with gap penalties (gap open = -400 and gap extend = 0). Pairwise sequence comparison was also carried out using sequence demarcation tool (Muhire *et al*., 2014). Maximum Likelihood phylogeny was predicted for the isolates under study that grouped into the genus *Luteovirus* computed with MEGA 5.1 (Tamura *et al.*, 2011) with best-fit nucleotide substitution (GTR+G) model. Sequence gaps were treated as complete deletion. Branch support of 3000 bootstrap iterations was used.

Isolate descriptors are shown accordingly as: [<isolate identifier>-<isolation host written as first word of genus name. full name of species>-<four-digit year-of-sampling following Gregorian calendar>-< country of sampling given as three-letter code defined in ISO 3166-1 alpha-3>].

RESULTS AND DISCUSSION

Initially, coat protein based PCR amplification followed by restriction digestion has been used for luteovirids classification (Robertson *et al.*, 1991). Both nucleotide

sequence along with serotype specificity were then used to classify and name different virus species. Commonly, amino acid discrimination of any gene product (more than 10% differences) criterion is used to classify luteoviruses (D'Arcy *et al.*, 2005). One gene product (for instance - CP) that may cause confusion of distinguishing one isolate to the rest of the virus group, however, may not be sufficient for discriminating virus species (Domier *et al.*, 2002). Therefore, there is a need to classify the virus on the basis of full genome sequences. The full genome sequence corresponding to a biological entity is more meaningful regarding biological information than an individual ORF, even if other features like plant-host specificity and serotype are known.

The divergence of >10% amino acids in any ORF, may not be indicative of species distinction. Percent identities of the luteoviral isolates, retrieved from the GenBank, do not follow amino acid divergence rule of >10% in several ORFs (Supplementary Table 1). For instance, the type member 'PS1' of BYDV-MAV shares only 88.8% amino acid identities in RTD with other MAV isolates. The three isolates '129', '064' and '0109' are the recognized members of PAS. However, the isolate '0109' shares only 88.2% and 86.2% amino acid identities (divergence of >10% amino acids in any ORF) in RTD with the isolates 'PAV-129' and '064', respectively (data not shown). Some isolates in PAV2 share less than 90% identities (i.e. 86.0–100% in CP, 84.3-100% in MP and 88.4-99.8% in RTD) among them. In RdRP, some isolates of PAV1 group share less identities (i.e. 87.7-99.8%) among them. Similarly, in RTD, PAS shares 86.2-94.7% identities, PAV1 has 88.3-99.6% identities, SbDV1 has 84.1-100% and SbDV2 has 84.9-99.8% within species (Supplementary Table 1). The divergence from the classification rule may be due to increase in the number of isolates identified in recent years for comparative studies or may be due to exhaustive nucleotide substitution rate.

Our analysis using 93 full-length isolates [from a total of 94 entities i.e. ruling out one contentious isolate (05YL5, Acc. No. EU332317); the outlier] of the genus *Luteovirus*, suggested a value of 71% species cut-off (Fig. 2, Table 3). Genome based nucleotide-based species cutoff (71%) divides all luteoviruses into five groups. Importantly, *PAV*, *PAS*, and *MAV* have been grouped into a single species, referred here as "Barley yellow dwarf virus". Similarly, *kerII* and *kerIII* were grouped together as members of one species "Barley yellow dwarf Kerguelen virus (BYDKV)". In accordance with the above, the phylogenetic analysis (Figure 1) showed *MAV*, *PAV* and *PAS* as closely related and in tight clustering. Similarly, *kerII* and *kerIII* were grouped together (Fig. 1) suggesting them as members of a single species.

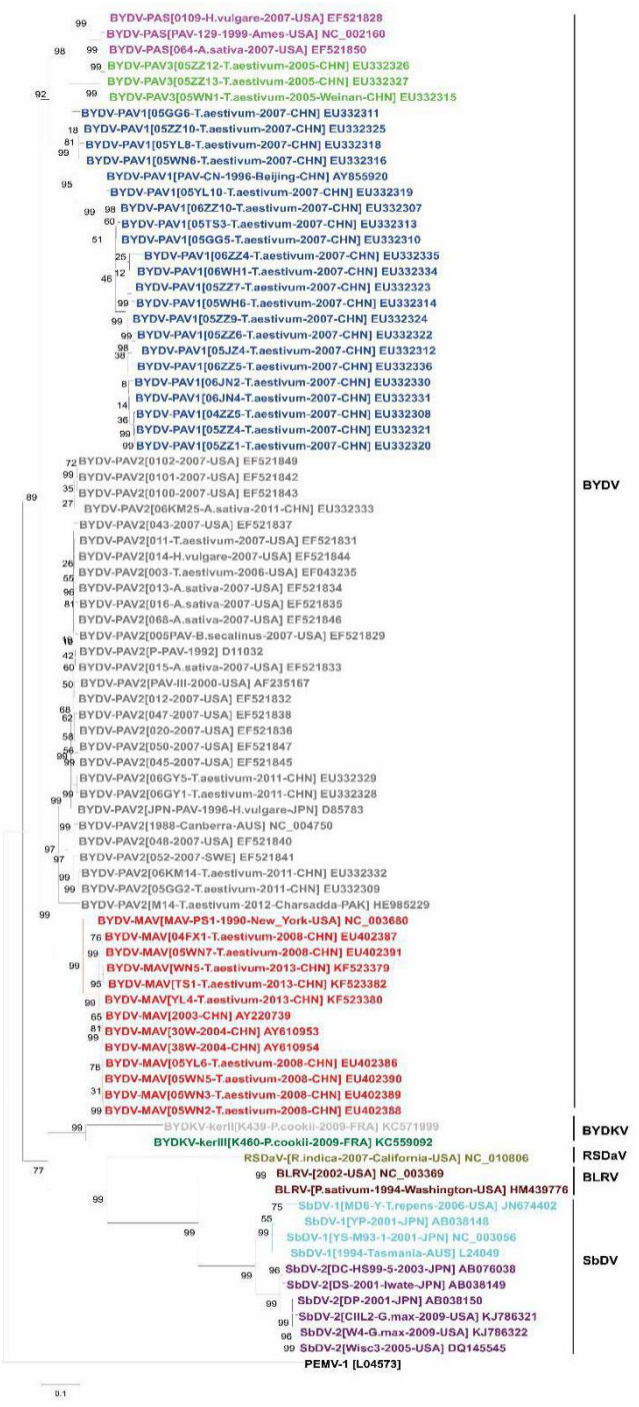
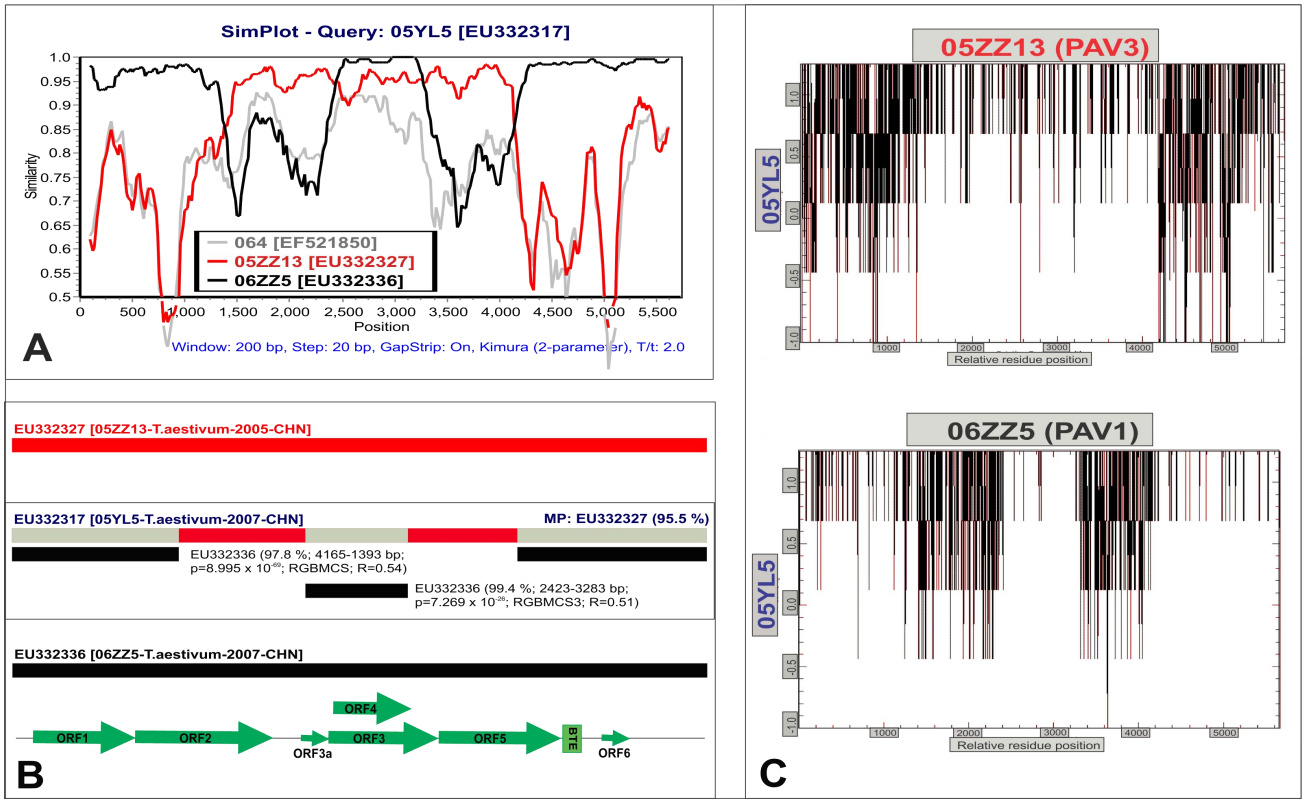


Figure 1. Phylogenetic tree based on the available full-genome *Luteovirus* sequences in the GenBank. The sequences were aligned with MUSCLE algorithm, and the evolutionary history was inferred with Maximum likelihood (ML) method, based on Tamura-Nei model, implemented in MEGA5 with 3000 Bootstrap replicates shown in percentage (Tamura *et al.*, 2011). *Luteovirus* isolates separated into clusters (probable strains) are shown with different colors. Suggested species groups are also indicated.



Supplementary Figure 1. Recombination analysis of BYDV-PAV isolate 05YL5 [GenBank: EU332317] A) Simplot analysis showing recombination breakpoints between BYDV-PAV1 isolate 06ZZ5 and BYDV-PAV3 isolate 05ZZ13. B) Recombination detection program 4 (RDP4) showing recombination detection between the two isolates 06ZZ5 and 05ZZ13 attested by high recombination score, percent similarity of each parents, number of detection algorithms being used and high p values. C) Plotcon (EMBOSS) analysis showing similarity matrix comparing the isolates 06ZZ5 and 05ZZ13 with the potential recombinant (isolate 05YL5).

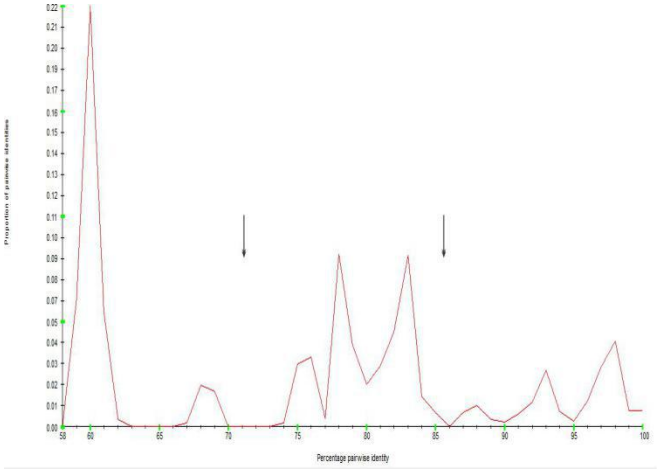


Figure 2. Distribution of full genome pairwise nucleotide sequence identity scores corresponding to 94 full length luteoviruses isolates available in the GenBank. The troughs / valleys at 71% and 86% are indicated.

In order to know either luteoviruses can better be classified at a strain level, a total of 94 full-length isolates were analyzed. By excluding two outliers, that are 05YL5 (Acc. No. EU332317) and ASL-1 (Acc. No. AJ810418), a cutoff (86%) classify luteoviruses into eleven strains as BYDV-MAV, BYDV-PAS, BYDV-PAV1, BYDV-PAV2, BYDV-PAV3, BYDKV-kerII, BYDKV-kerIII, RSDaV, BLRV, SbDV-1 and SbDV-2. Specifically, the new cutoff for strain further divided the known *PAV* isolates into three strains as PAV1, PAV2 and PAV3. The *Luteovirus* isolates, in general, do not correspond to geographical distribution, specifically, the PAV2 – a large sub-clade – contained isolates from America, Australia, China, Japan, Pakistan, and Sweden. In contrast, relating to this geographic origin heterogeneity, the sub-clade PAS was only constituted by isolates of American origin, and the two other subclades PAV1 and PAV3 constituted only by isolates of Chinese origin (Figure 1). Similarly, the suggested species divide SbDV into SbDV-1 and SbDV-2. Ironically, there is a need to characterize the

new strains biologically, in the future, in order to warrant their significance in classification.

The demarcation cutoff values were supported by the pairwise distance distribution plot (Fig. 2) in which clear valleys (troughs) at 71% (for species) and 86% (for strain) were prominent. The two isolates 05YL5 and ASL-1, may be due to homologous recombination were highly related to several species that make the classification a bit difficult at one threshold. Three independent recombination detection programs – Simplot (Lole *et al*., 1999), Plotcon (Rice *et al*., 2000) and RDP4 (Martin *et al.*, 2015) showed that the isolate 05YL5 is a chimeric genome with the isolate 05ZZ13 (PAV3) as major parent and the isolate 06ZZ5 (PAV1) as a minor parent (Supplementary Figure 1). Similarly, the other outlier ASL1-1 is also a probable recombinant of two PAV2 isolates, M14 and 06KM25 (data not shown).

Based on the analysis, five species were identified that were further categorized into eleven strains (Table 2, 4). By implementing the working cutoff value (71% for species and 86% for strain), two outliers (isolate 05YL5 and ASL-1) were observed belonging to two (or more) distinct species. The conflict may be resolved by considering each of them belonging to the group that includes the isolate(s) with which they share highest percentage pairwise identity. Therefore, 05YL-5 may be considered as a member of PAV1 that showed maximum identity of 92.5 (data not shown) with isolate 06ZZ5. Whereas, the other conflicting isolate ASL-1, showing maximum identity of 93.2% (data not shown) with isolate Aus [NC_004750], may thus be considered as a member of PAV2.

Serological differences and divergence of >10% amino acids in any ORF, indicate species distinction. However, for species demarcation, inference from the full length sequences is more meaningful, if considered, since they correspond to biological entities and may represent occurrence of genetic recombination. Pairwise sequence comparison carried out, in this study, using sequence demarcation tool (Muhire *et al.*, 2014), has previously been used for *Mastrevirus* (Muhire *et al.*, 2013), *Curtovirus* (Varsani *et al.*, 2014) and more recently for *Begomovirus* (Brown *et al.*, 2015) of the family *Geminiviridae*. Sequence based species cutoff alone is accurate and reflects biological differences between viruses belonging to different species (Brown *et al.*, 2015) at least in the case of *Begomovirus* [Family Geminiviridae] – *Bean golden mosaic virus* (phloem restricted) and *Bean golden yellow mosaic virus* (invades mesophyll tissues). In addition, phylogenetic trees that represent gene tree instead of a virus tree can create misconceptions about viral genome structure and can lead to incorrect evolutionary inferences.

Table 2. Summary of the suggested species and strains within the genus *Luteovirus* based on full length genome nucleotide identities.

Five suggested species based on 71% cutoff		**Eleven suggested strains based on 86 % cutoff**	
1	Barley yellow dwarf virus	1	Barley yellow dwarf virus - MAV
		2	Barley yellow dwarf virus – PAS
		3	Barley yellow dwarf virus - PAV1
		4	Barley yellow dwarf virus - PAV2
		5	Barley yellow dwarf virus - PAV3
2	Barley yellow dwarf Kerguelen virus	6	Barley yellow dwarf Kerguelen virus - kerII
		7	Barley yellow dwarf Kerguelen virus kerIII
3	Rose spring dwarf-associated virus	8	Rose spring dwarf-associated virus
4	Bean leaf roll virus	9	Bean leaf roll virus
5	Soybean dwarf virus	10	Soybean dwarf virus – 1
		11	Soybean dwarf virus – 2

Table 3. Nucleotide identities of full length luteovirus genomes grouped into five species suggested based on 71% cutoff.

	SbDV (17)	**BYDKV (02)**	**RSDaV (01)**	**BLRV (02)**	**BYDV (71)**
BYDV (71)	58.2-62.2	67.1-69.1	59.7-61.8	58.4-61.7	74.1-100
BLRV (02)	67.6-69.3	59.6-60.8	61.7-62.3	97.3	
RSDaV (01)	60.4-61.8	60.2-60.8	**		
BYDKV(02)	58.8-60.8	71.5			
SbDV (17)	81.4-100				

Table 4. Summary of the isolates assigned to the suggested *Luteovirus* strains.

Sr.	Virus Strain	GenBank	Full length description of the isolates
1	Barley yellow dwarf virus – MAV	NC_003680	[PS1-1990-New_York-USA]
		AY220739	[05WN5-T.aestivum-2008-CHN]
		EU402390	[05WN3-T.aestivum-2008-CHN]
		EU402389	[05WN2-T.aestivum-2008-CHN]
		EU402388	[04FX1-T.aestivum-2008-CHN]
		EU402387	[05YL6-T.aestivum-2008-CHN]
		EU402386	[38W-2004-CHN]
		AY610954	[30W-2004-CHN]
		AY610953	[YL4-T.aestivum-2013-CHN]
		KF523380	[05WN7-T.aestivum-2008-CHN]
		EU402391	[WN5-T.aestivum-2013-CHN]
		KF523379	[2003-CHN]
		KF523382	[TS1-T.aestivum-2013-CHN]
2	Barley yellow dwarf virus – PAS	NC_002160	[PAV-129-1999-Ames-USA]
		EF521850	[064-A.sativa-2007-USA]
		EF521828	[0109-H.vulgare-2007-USA]
3	Barley yellow dwarf virus - PAV1	AY855920	[PAV-CN-1996-Beijing-CHN]
		EU332319	[05YL10-T.aestivum-2007-CHN]
		EU332316	[05WN6-T.aestivum-2007-CHN]
		EU332310	[05GG5-T.aestivum-2007-CHN]
		EU332307	[06ZZ10-T.aestivum-2007-CHN]
		EU332313	[05TS3-T.aestivum-2007-CHN]
		EU332325	[05ZZ10-T.aestivum-2007-CHN]
		EU332318	[05YL8-T.aestivum-2007-CHN]
		EU332311	[05GG6-T.aestivum-2007-CHN]
		EU332335	[06ZZ4-T.aestivum-2007-CHN]
		EU332321	[05ZZ4-T.aestivum-2007-CHN]
		EU332331	[06JN4-T.aestivum-2007-CHN]
		EU332308	[04ZZ5-T.aestivum-2007-CHN]
		EU332320	[05ZZ1-T.aestivum-2007-CHN]
		EU332323	[05ZZ7-T.aestivum-2007-CHN]
		EU332324	[05ZZ9-T.aestivum-2007-CHN]
		EU332314	[05WH6-T.aestivum-2007-CHN]
		EU332336	[06ZZ5-T.aestivum-2007-CHN]
		EU332330	[06JN2-T.aestivum-2007-CHN]
		EU332322	[05ZZ6-T.aestivum-2007-CHN]
		EU332334	[06WH1-T.aestivum-2007-CHN]
		EU332312	[05JZ4-T.aestivum-2007-CHN]
4	Barley yellow dwarf virus - PAV2	NC_004750	[1988-Canberra-AUS]
		HE985229	[M14-T.aestivum-2012-PAK]
		EU332329	[06GY5-T.aestivum-2011-CHN]
		EU332328	[06GY1-T.aestivum-2011-CHN]
		EU332333	[06KM25-A.sativa-2011-CHN]
		D11032	[P-PAV-1992]
		EU332332	[06KM14-T.aestivum-2011-CHN]
		EU332309	[05GG2-T.aestivum-2011-CHN]
		AF235167	[PAV-Ill-2000-USA]
		EF521841	[052-2007-SWE]
		EF521844	[014-H.vulgare-2007-USA]
		EF521838	[047-2007-USA]
		EF521836	[020-2007-USA]
		EF521837	[043-2007-USA]
		EF521832	[012-2007-USA]
		EF521846	[068-A.sativa-2007-USA]
		EF043235	[003-T.aestivum-2006-USA]
		EF521847	[050-2007-USA]
		EF521845	[045-2007-USA]
		EF521840	[048-2007-USA]

Sr.	Virus Strain	GenBank	Full length description of the isolates
		EF521834	[013-A.sativa-2007-USA]
		EF521831	[011-T.aestivum-2007-USA]
		EF521829	[005PAV-B.secalinus-2007-USA]
		EF521849	[0102-2007-USA]
		EF521843	[0100-2007-USA]
		EF521835	[016-A.sativa-2007-USA]
		EF521833	[015-A.sativa-2007-USA]
		EF521842	[0101-2007-USA]
		D85783	[JPN-PAV-1996-H.vulgare-JPN]
5	Barley yellow dwarf virus - PAV3	EU332326	[05ZZ12-T.aestivum-2005-CHN]
		EU332327	[05ZZ13-T.aestivum-2005-CHN]
		EU332315	[05WN1-T.aestivum-2005-Weinan-CHN]
6	Barley yellow dwarf Kerguelen virus – kerII	KC571999	[K439-P.cookii-2009-FRA]
7	Barley yellow dwarf Kerguelen virus – kerIII	KC559092	[K460-P.cookii-2009-FRA]
8	Rose spring dwarf-associated virus	NC_010806	[R.indica-2007-California-USA]
9	Bean leaf roll virus	NC_003369	[2002-USA]
		HM439776	[P.sativum-1994-Washington-USA]
10	Soybean dwarf virus – 1	L24049	[1994-Tasmania-AUS]
		JN674402	[MD6-Y-T.repens-2006-USA]
		NC_003056	[YS-M93-1-2001-JPN]
		AB038148	[YP-2001-JPN]
11	Soybean dwarf virus – 2	KJ786322	[W4-G.max-2009-USA]
		KJ786321	[ClIL2-G.max-2009-USA]
		DQ145545	[Wisc3-2005-USA]
		AB076038	[DC-HS99-5-2003-JPN]
		AB038150	[DP-2001-JPN]
		AB038149	[DS-2001-Iwate-JPN]

A virus isolate, according to van Regenmortal, is an instance of a particular virus (Kuhn *et al.*, 2013). However, naming every instance of a *Luteovirus* passaged (in their host plants) in the lab as a separate isolate may not be suggested. Thus, seven passaged instances of SbDV isolate MD6-Y were not considered in Figure 1 and Table 4. Furthermore, a virus is a real thing and is static. Whereas, a species is an idea or concept and may not stand the test of time (Kuhn *et al.*, 2013). It is, therefore, needed to differentiate the virus from the concept of species, has been left for the future studies. In addition to the above, there is also a need to consider the nomenclature of the virus for consistency, along with the classification, however, is beyond the scope of this article.

***Conclusions*:** According to the ninth report of International Committee on Taxonomy of Viruses (ICTV), the virus isolates belonging to the genus *Luteovirus* have been grouped into eight species, recognized based on the breath and specificity of host range, serology and >10% amino acid divergence any ORF. Critically, luteoviruses transmission cannot be linked with their antigenic features (serotype). Furthermore, as a consequence of maritime trade among different countries in the past, a major hypothesis, frequent recombinant genomes of the members of *Luteoviridae* have been reported. Mainly, because of the cluster class, one gene product (amino acid sequence), will not accommodate occurrence of recombination, may not be sufficient for species distinction. Also, single ORF may not represent or may create misconception about the viral genome structure. Full genome sequence, which corresponds to a virus entity, is thus necessary for taxonomic purposes, diversity studiesor disease epidemiology. By analyzing viral complete genome sequence identities, we suggest, tentatively, five species (cutoff 71%) and eleven strains (cutoff 86%) in the genus *Luteovirus*. The suggested species, group *MAV*, *PAV* and *PAS* into a single species termed as BYDV species. Similarly, *kerII* and *kerIII* have been grouped together as members of a suggested species, BYDKV.

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