



# Nanovirus Disease Complexes: An Emerging Threat in the Modern Era

Aamir Lal<sup>1</sup>, Thuy Thi Bich Vo<sup>1</sup>, I Gusti Ngurah Prabu Wira Sanjaya<sup>1</sup>, Phuong Thi Ho<sup>1</sup>, Ji-Kwang Kim<sup>2</sup>, Eui-Joon Kil<sup>1,3\*</sup> and Sukchan Lee<sup>1\*</sup>

<sup>1</sup> Department of Integrative Biotechnology, Sungkyunkwan University, Suwon, South Korea, <sup>2</sup> Research and Development Bureau, Chungcheongnam-do Agricultural Research and Extension Services, Yesan, South Korea, <sup>3</sup> Department of Plant Medicals, Andong National University, Andong, South Korea

## OPEN ACCESS

### Edited by:

Toufic Elbeaino,  
International Centre for Advanced  
Mediterranean Agronomic Studies,  
Italy

### Reviewed by:

Stéphane Blanc,  
Institut National de la Recherche  
Agronomique de Montpellier, France  
Ana Grande-Pérez,  
Institute of Subtropical  
and Mediterranean Horticulture “La  
Mayora”, Spain  
Heiko Ziebell,  
Julius Kühn-Institut, Germany

### \*Correspondence:

Eui-Joon Kil  
viruskil@anu.ac.kr  
Sukchan Lee  
cell4u@skku.edu

### Specialty section:

This article was submitted to  
Plant Pathogen Interactions,  
a section of the journal  
Frontiers in Plant Science

**Received:** 02 May 2020

**Accepted:** 28 October 2020

**Published:** 19 November 2020

### Citation:

Lal A, Vo TTB, Sanjaya IGPNW,  
Ho PT, Kim J-K, Kil E-J and Lee S  
(2020) Nanovirus Disease Complexes:  
An Emerging Threat in the Modern  
Era. *Front. Plant Sci.* 11:558403.  
doi: 10.3389/fpls.2020.558403

Multipartite viruses package their genomic segments independently and mainly infect plants; few target animals. *Nanoviridae* is a family of multipartite single-stranded DNA plant viruses that individually encapsidate single-stranded DNAs of approximately 1 kb and transmit them through aphids without replication in the aphid vectors, thereby causing important diseases of leguminous crops and banana. Significant findings regarding nanoviruses have recently been made on important features, such as their multicellular way of life, the transmission of distinct encapsidated genome segments through the vector body, evolutionary ambiguities, mode of infection, host range and geographical distribution. This review deals with all the above-mentioned features in view of recent advances with special emphasis on the emergence of new species and recognition of new host range of nanoviruses and aims to shed light on the evolutionary linkages, the potentially devastating impact on the world economy, and the future challenges imposed by nanoviruses.

**Keywords:** nanoviruses, multipartite virus, evolution, host range, geographical distribution, geminiviruses

## INTRODUCTION

Among viruses, single-stranded (ss) DNA viruses are a considerable threat to all living organisms. These ssDNA viruses infect both plants and animals. Circoviruses (Tischer et al., 1986; Cheung, 2006), bidensovirus (Hayakawa et al., 2000), small circular (smaco) viruses (Ng et al., 2015), redondovirus (Abbas et al., 2019), anelloviruses (Biagini et al., 2006; Blatter et al., 2018), genomovirus (Krupovic et al., 2016), and circular replication-associated protein (Rep)-encoding single-stranded (CRESS) DNA viruses (Dayaram et al., 2014; Rosario et al., 2015) are some of the important ssDNA viruses, which infect animals, silkworm, human beings, fungi, insects and marine invertebrates, respectively. ssDNA viruses are largely known for their devastating effects on the plant world (Goodman, 1977; Kenyon et al., 2014; Malathi and Renuka Devi, 2019b). Among these ssDNA viruses, monopartite and bipartite viruses, with one and two segments, respectively, are very common. In these viruses, nucleic acid segments are encapsidated into a single virion (viral particle) which propagates as a whole. Some viruses are multipartite and have two or more segmented genomes packaged into separate virions, each of them capable of propagating independently (Randles et al., 2000; Sicard et al., 2016).

Based on their genomic organization, the International Committee on the Taxonomy of Viruses (ICTV) categorized ssDNA plant viruses into two families: (i) *Geminiviridae* (Zerbini et al., 2017) and (ii) *Nanoviridae* (Randles et al., 2000; Vetten et al., 2012). *Geminiviridae* is the largest family of plant viruses which can infect a large number of hosts belonging to several plant genera and

families. *Nanoviridae*, the focal point of this study, comprises plant viruses possessing very small virions which contain a multipartite (6–8), circular, single stranded DNA genome of approximately 1 kb in length, along with a few satellite molecules, each possessing a specific function (Vetten et al., 2012; Briddon et al., 2018; Malathi and Dasgupta, 2019a). Highly diversified host ranges are ascribed to *Nanoviridae* members which induce symptoms such as stunting, dwarfism, necrosis, mosaic, and leaf rolling in host plants and may eventually lead to plant death as well (Mandal, 2010; Grigoras et al., 2014; Hull, 2014; Gaafar et al., 2017, 2018). Viral replication occurs in the nucleus of infected cells via ssDNA rolling circle amplification (Rosario et al., 2012; Jeske, 2018). In addition, aphid transmission is a key characteristic for viruses belonging to the *Nanoviridae* family (Sano et al., 1998; Franz et al., 1999). Here we outline the *Nanoviridae* family and delve into the recent developments while identifying its impact on the agricultural world.

## **NANOVIRIDAE FAMILY: CLASSIFICATION, GENOMIC-STRUCTURE, AND FUNCTION**

The *International Committee on Taxonomy of Viruses (ICTV)* categorized the *Nanoviridae* family into two genera, *Nanovirus* and *Babuvirus*, based on their genome organization and transmission vectors, along with categorization of coconut foliar decay virus (CFDV) as an unassigned species (Mandal, 2010; **Table 1**). Nanoviruses are non-enveloped with icosahedral and round geometries, and  $T = 1$  symmetry with a diameter of 18–19 nm (**Figures 1A,B**). Contrary to geminiviruses, nanoviruses are multipartite viruses with 8–10 circular ssDNA components of approximately 1 kb in size (Sano et al., 1998; Gronenborn, 2004), while babuviruses contain six components with a size of approximately 1–1.1 kb (Halbert and Baker, 2015) and 12 DNAs of approximately 1.2–1.4 kb in size in association with CFDV (Gronenborn et al., 2018). Additional circular, ssDNA molecules (~1–1.4 kb) that encode Rep protein, referred to as satellite molecules, have also been reported along with nanoviruses and babuviruses recently and categorized as *nano alphasatellites*. Those in the unassigned CFDV are categorized into unassigned species in the family *alphasatellitidae* (Briddon et al., 2018).

Whilst the genomes of geminiviruses are encoded by one or two circular ssDNA molecules, the genomes of *Nanoviridae* members are encoded by six or eight components (**Figure 1B**). Furthermore, these components are encapsidated separately into individual virions each with a specific role (Harrison, 1985; Randles et al., 2000; Saunders et al., 2003). DNA R encodes the master replication (M-Rep) initiator protein (Timchenko et al., 2000; Horser et al., 2001), DNA M encodes the movement protein, DNA C encodes the cell-cycle-link (clink) protein (Aronson et al., 2000), DNA S encodes the capsid protein (CP) (Wanitchakorn et al., 1997), and DNA N encodes the nuclear shuttle protein (NSP) (Wanitchakorn et al., 2000; Gronenborn, 2004) (Function of CP, Rep and NSP explained in

following sections). Despite the numerous attempts to investigate U1, U2, and U4 of nanoviruses and U3 of babuviruses as well as the satellite molecules associated with nanoviruses, their biological functions remain unclear (**Figures 2A–C**).

## **GEOGRAPHICAL DISTRIBUTION OF NANOVIRIDAE MEMBERS**

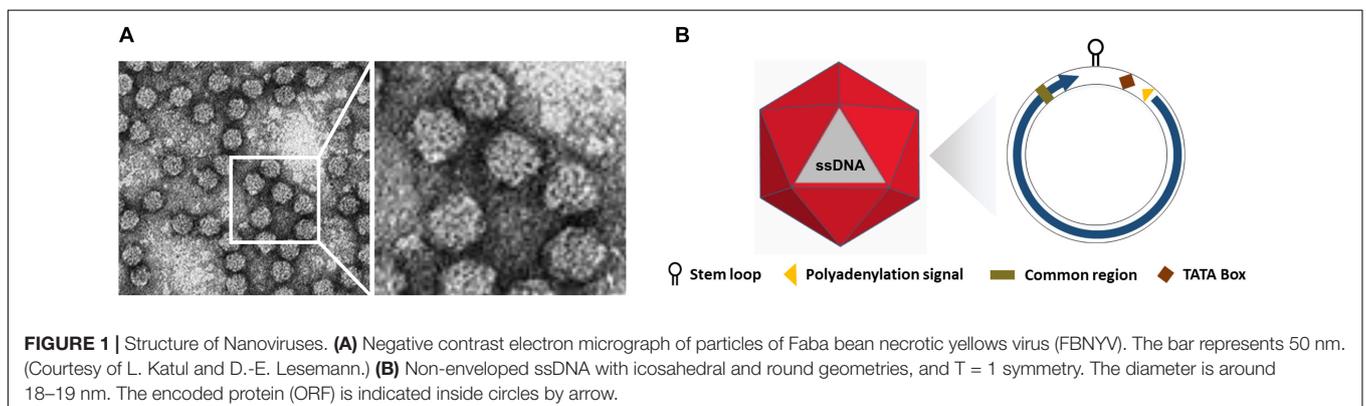
There have been increasing reports of the presence of *Nanoviridae* members from different regions of the world. Between the genera of the family *Nanoviridae*, babuviruses are highly ubiquitous viruses, e.g., banana bunchy top virus (BBTV) has been reported almost throughout the world (Sun, 1961; Burns et al., 1995; Beetham et al., 1997; Amin et al., 2008; Almeida et al., 2009; Blomme et al., 2013). Abaca bunchy top virus (ABTV) and cardamom bushy dwarf virus (CBDV) are found in the Philippines and Malaysia (Sharman et al., 2008) and India, respectively (Mandal et al., 2004; Ghosh et al., 2015). *Nanoviridae* members have marked their presence in major continents: Asia, Europe, Africa, and Australia. Among these nanoviruses, some were observed to be limited to certain areas or particular countries within a continent, while some exhibited high diversity through their presence across many continents, such as cow vetch latent virus (CVLV) in France; sophora yellow stunt-associated virus (SYSaV) and milk vetch chlorotic dwarf virus (MVCDV) in Iran; and faba bean yellow leaf virus (FBYLV) reported only in Ethiopia (Abraham et al., 2012; Heydarnejad et al., 2017; Hassan-Sheikhi et al., 2020); milk vetch dwarf virus (MDV) and subterranean clover stunt virus (SCSV) in Asia (Boevink et al., 1995; Sano et al., 1998; Lal et al., 2018); pea necrotic yellow dwarf virus (PNYDV), black medic leafroll virus (BMLRV), parsley severe stunt associated virus (PSSaV) and pea yellow stunt virus (PYSV) in Europe (Grigoras et al., 2014; Gaafar et al., 2016; Vetten et al., 2019); faba bean necrotic yellows virus (FBNYV) and faba bean necrotic stunt virus (FBNSY) in Asia, Europe, and Africa (Katul et al., 1998; Grigoras et al., 2010b); and CFDV unassigned species detected from Vanuatu, located near the South Pacific Ocean (Gronenborn et al., 2018; **Figure 3**). Interestingly, no nanoviruses have been identified in the new world to date. Though some of these viruses could be invasive, while others may have been there for ages and have been increasingly identified in these regions due to an increasing number of metagenomic studies (Gaafar et al., 2018; Gronenborn et al., 2018; Vetten et al., 2019; Hassan-Sheikhi et al., 2020; Lal et al., 2020), data scarcity confines our analysis. The purpose of listing the species at different locations around the world is simply to reflect the regions in which the specific nanoviruses were identified.

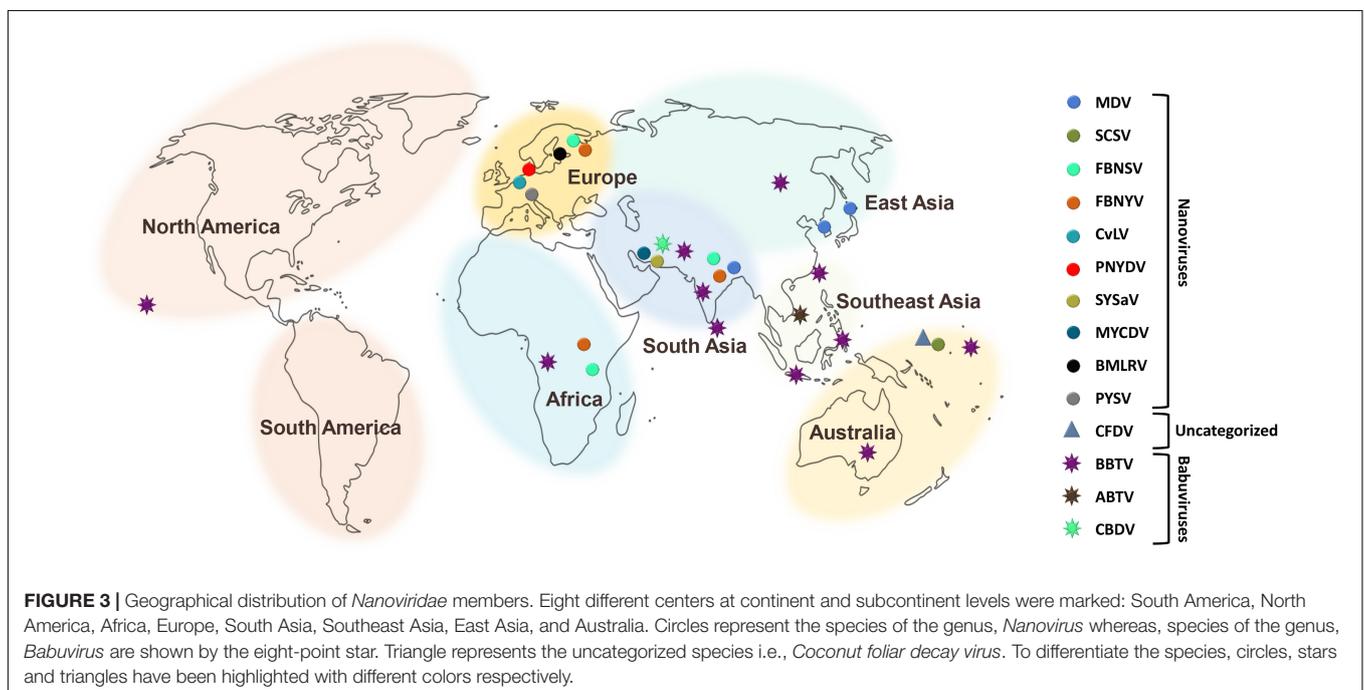
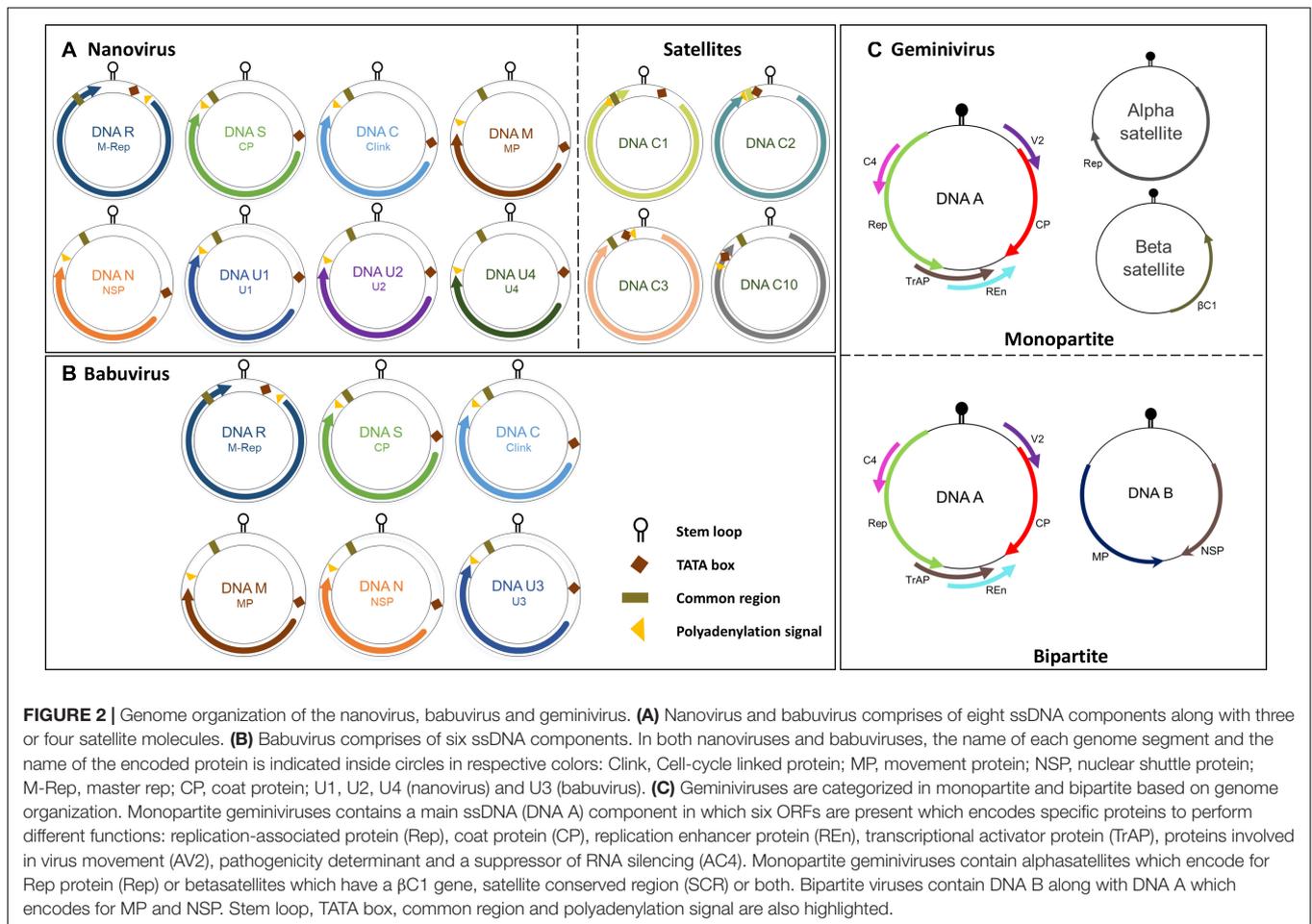
## **HOST RANGE AND SYMPTOMS**

Nanoviruses are considered as viral agents with limited host range. Among nanoviruses, babuviruses infect only the monocot species, *Musaceae* and *Zingiberaceae* (Burns et al., 1995; Mandal et al., 2004; Amin et al., 2008). No other plant families

**TABLE 1** | Introduction to *Nanoviridae*: occurrence, transmission, host ranges and symptoms development.

Genus	Species	Family	Host Species	Symptoms	Transmission	References
<b>Nanovirus</b>	<i>Subterranean clover stunt virus</i> (SCSV)	Fabaceae	<i>Trifolium subterraneum</i> <i>Medicago hispida</i> <i>Macroptilium lathyroides</i>	Chlorosis and stunting	<i>Aphis craccivora</i> , <i>A. gossypii</i> ,	Boevink et al., 1995
	<i>Faba bean necrotic yellows virus</i> (FBNYV)	Fabaceae	<i>Cicer arietinum</i> , <i>Vicia faba</i> <i>Phaseolus vulgaris</i>	Necrosis and leaf rolling	<i>Acyrtosiphon pisum</i>	Katul et al., 1998
	<i>Faba bean necrotic stunt virus</i> (FBNSY)	Fabaceae	<i>Lens culinaris</i> <i>Vicia sativa</i>	Necrosis and stunting	<i>Acyrtosiphon pisum</i> <i>Aphis craccivora</i>	Grigoras et al., 2010a
	<i>Pea necrotic yellow dwarf virus</i> (PNYDV)	Fabaceae	<i>Pisum sativum</i> <i>Vicia faba</i> , <i>V. sativa</i> and <i>Lens culinaris</i>	Stunting, dwarfing, yellowing and leaf rolling	<i>Acyrtosiphon pisum</i>	Gaafar et al., 2016
	<i>Milk Vetch Dwarf Virus</i> (MDV)	Fabaceae Caricaceae Solanaceae	<i>Astragalus sinicus</i> <i>Glycine max</i> <i>Carica papaya</i> <i>Solanum lycopersicum</i> <i>Capsicum annuum</i>	Stunting, dwarfing, vein yellowing	<i>Aphis craccivora</i>	Sano et al., 1998
	<i>Faba bean yellow leaf virus</i> (FBYLV)	Fabaceae	<i>Vicia faba</i>	Yellowing, stunting, necrosis and leaf deformation	<i>Acyrtosiphon pisum</i>	Abraham et al., 2012
	<i>Black medic leaf roll virus</i> (BMLRV)	Fabaceae	<i>Medicago lupulina</i> <i>Pisum sativum</i>	Leaf rolling	–	Grigoras et al., 2014
	<i>Pea yellow stunt virus</i> (PYSV)	Fabaceae	<i>Pisum sativum</i>	Stunting, yellowing	–	Grigoras et al., 2014
	<i>Cow vetch latent virus</i> (CvLV)	Fabaceae	<i>Vicia cracca</i>	–	–	Gallet et al., 2018
	<i>Sophora yellow stunt associated virus</i> (SYSaV)	Fabaceae	<i>Sophora alopecuroides</i> L.	Dwarfing, yellowing, stunted leaves and yellow vein banding.	–	Heydarnejad et al., 2017
	<i>Parsley severe stunt associated virus</i> (PSSaV)	Apiaceae	<i>Petroselinum crispum</i> (Mill.) Fuss	Stunting, leaf yellowing and leaf curling.	–	Vetten et al., 2019
	<i>Milk vetch chlorotic dwarf virus</i> (MVCDV)	Fabaceae	<i>Astragalus myriacanthus</i> Boiss.	Leaf chlorosis, little leaves and dwarfism	–	Hassan-Sheikhi et al., 2020
<b>Babuvirus</b>	<i>Banana bunchy top virus</i> (BBTV)	Musaceae	<i>Musa</i> spp.	Dark green streaks plant stunting	<i>Pentalonia nigronervosa</i>	Burns et al., 1995
	<i>Abaca bunchy top virus</i> (ABTV)	Musaceae	<i>Musa</i> spp.	Mosaic	–	Sharman et al., 2008
	<i>Cardamom bushy dwarf virus</i> (CBDV)	Zingiberaceae	<i>Amomum subulatum</i>	Streak mosaic Bushy appearance	<i>Micromyzus-kalimpongensis</i>	Mandal et al., 2004
<b>Coconut foliar decay virus</b>	<i>Coconut foliar decay virus</i> (CFDV)	Areaceae	<i>Cocos nucifera</i>	foliar decay	<i>Myndus tiffany</i>	Gronenborn et al., 2018

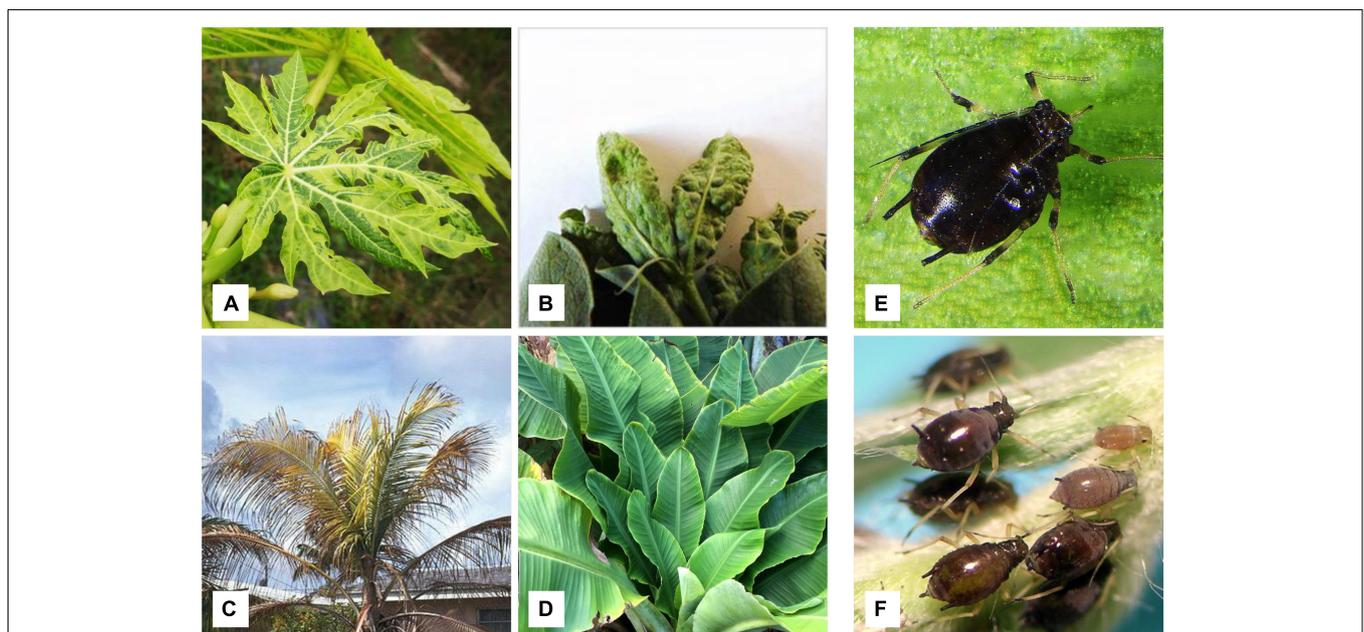




have been reported to be infected by babuviruses. BBTV mainly infects *Musa acuminata*, *M. coccinea*, *M. balbisiana*, *M. ornata*, *M. jackeyi*, *M. textilis*, and *M. velutina* (Burns et al., 1995; Sharman et al., 2008; Qazi, 2016). Nanoviruses were considered to affect only the legumes (Franz et al., 1997; Abraham et al., 2010; Grigoros et al., 2010a). Fabaceae, also known as Leguminosae, a legume family, is an ideal target for infection by nanovirus members (Abraham et al., 2010, 2012; Grigoros et al., 2014). About 50 Fabaceae species are infected by these members, and this number continues to increase (Franz et al., 1997; Gaafar et al., 2016) (nanoviruses with respective host ranges are listed in detail in **Table 1**). Nanoviruses limitation to narrow host ranges was a major factor in considering them as low impact viruses with an exiguous domain. This is why geminiviruses with the infection severity and outbreaks in broad host range have always been a preferred research area among ssDNA viruses compared to nanoviruses (Harrison, 1985; Mansoor et al., 2003; Jeske, 2009; Kenyon et al., 2014; Kil et al., 2016; Rodrigues et al., 2019). Recent developments have contributed to the discovery of new nanovirus hosts by confirming their presence in various important plant families including both dicots as well as monocots. For example, MDV was recently reported in dicots families i.e., Caricaceae (*Carica papaya*) (Lal et al., 2018), Solanaceae (*Solanum lycopersicum*, *Capsicum annuum*) (Lal et al., 2020) and in monocots family i.e., Liliaceae (*Lilium candidum*) (Lal et al., 2018). Moreover, the unassigned species, CFDV has been reported in a monocot family i.e., Arecaceae (*Cocos nucifera*) (Gronenborn et al., 2018). Recent identification

of new nanoviruses in new host plants is an intriguing aspect to be focused on. PSSaV was recently reported in Apiaceae [*Petroselinum crispum* (Mill.) Fuss] (Vetten et al., 2019) whereas, MVCDV was reported in Fabaceae (*A. myriacanthus* Boiss) (Hassan-Sheikhi et al., 2020). Recent discoveries show the ongoing surge of viral infection evidence in various new host plant species owing to the growing number of metagenomics studies.

Generally, symptom development in nanovirid-infected plant species resembles that observed in *Geminiviridae* infections, such as chlorosis, necrosis, leaf rolling, dwarfing, stunting, leaf yellowing, vein yellowing, leaf deformation, and plant death (Mansoor et al., 2003; Spence et al., 2007; Jeske, 2009; Hull, 2014; Kenyon et al., 2014; Gaafar et al., 2016; Rodrigues et al., 2019; Saucke et al., 2019; Vetten et al., 2019; **Figures 4A–D**). However, *Geminiviridae* has a much more diverse assortment of associated symptoms. *Nanoviruses* induce almost all of the symptoms mentioned above in their respective hosts (**Table 1**). Each nanovirus has been named according to its major symptom; for example, FBNYV, MVCDV, MDV, and BMLRV show leaf yellowing, leaf chlorosis, dwarfism, and leaf rolling, respectively. Babuviruses also show a slight deviation in symptom development, inducing dark green streaks, streak mosaicism and a bushy appearance in hosts infected by the BBTV, ABTV, and CBDV, respectively. Coconut foliar decay is observed in the case of unassigned species CFDV (Merits et al., 2000). Mostly, clear symptoms can be observed in parts of the plant infected by any member of the *Nanoviridae* family. but in few recently reported cases,



**FIGURE 4** | Symptomatic host plants of nanovirus and babuviruses along with insect vectors. **(A)** Papaya plant showing leaf yellowing and dwarfism and found infected with MDV (nanovirus). **(B)** Faba bean showing necrosis and infected with FBNYV (nanovirus). **(C)** Coconut tree exhibiting foliar decay due to CFDV (uncategorized). **(D)** Banana plant showing bunchy top disease symptoms and infected with BBTV. Insect vectors, **(E)** *Pentalonía nigronervosa* transmits babuviruses and **(F)** *Aphis craccivora* responsible for the transmission of nanoviruses.

no significant symptoms were observed i.e., MDV infection in Solanaceous members (Lal et al., 2020). Nanoviruses do not exhibit any phenotypic difference with other viruses and no symptom can be associated specifically with the nanoviruses to date.

## DIVERGENCE OF NANOVIRIDAE FROM GEMINIVIRIDAE AND CIRCOCOVIRIDAE

*Nanoviridae* are more closely related to *Geminiviridae* and *Circoviridae* among the seven families of ssDNA viruses in the phylum Cressdnaviricota (Krupovic et al., 2020). *Geminiviridae* is one of the largest families of plant viruses belonging to the order *Geplafuvirales* of the *Repensiviricetes* class. Replication occurs via a rolling circle mechanism, highly conserved sequences TARTATTAC (geminiviruses), TANTATTAC (nanoviruses) in the loop of a putative stem-loop structure within the IR, and the association with satellite molecules (especially alphasatellites in) are common features of the plant virus families *Geminiviridae* and *Nanoviridae* (Burns et al., 1995; Sano et al., 1998; Timchenko et al., 2000; Buchmann et al., 2009). Based on these resemblances, the members of these viral families are considered cousin viruses (Koonin et al., 2015).

The family *Circoviridae* involves a community of diverse animal viruses with small, closed-circular, ssDNA that belongs to the order *Cirlivirales* of the *Arfiviricetes* class. *Nanoviridae* shares the same class but different order i.e., Mulpavirales with *Circoviridae*. Their genome size is ~1.7–2.1 kb and consists of two ORFs in the opposite direction with NANTATTAC as highly conserved sequences. These animal circoviruses are closely related to plant nanoviruses, as the Rep of circoviruses shows high similarity to the Rep of nanoviruses

(Simmonds et al., 2017). The origin of replication (ori) in both the circovirus and nanovirus DNA is adjacent to the N-terminal part of the Rep gene (Niagro et al., 1998). This similarity between circovirus and nanovirus ori sequences indicates that these sequences have evolved from a common ancestral sequence (Katul et al., 1998), and that the circovirus has evolved to infect a vertebrate in various intermediate stages over time (Gibbs and Weiller, 1999). Another study showed that Rep proteins of marine ssDNA viruses show high resemblance with nanoviruses. A high copy number viral genome has been isolated from an algal cell identifying protists as the possible origin of nanoviruses, circoviruses and geminiviruses (Yoon et al., 2011). Despite some common factors, *Nanoviridae* exhibit certain contradictions to *Geminiviridae* and *Circoviridae* in terms of their genome organization, way of transmission, mode of infection, host range, and symptoms development (see details in **Table 2**; Sano et al., 1998; Gronenborn, 2004; Sharman et al., 2008; Iranzo and Manrubia, 2012; Halbert and Baker, 2015; Sicard et al., 2015; Di Mattia et al., 2020).

## MULTICELLULAR WAY OF LIFE FOR NANOVIRIDAE

In viruses, multipartitism may exert benefits by conferring greater stability due to the genome compartmentalization of smaller-sized segments (Ojosnegros et al., 2011), by increasing the possibility of faster replication of small genomic segments (Nee, 1987), by generating non-mutated offspring (Pressing and Reanney, 1984), or by increasing genome shuffling (Chao, 1988, 1991). In contrast, multipartitism has drawbacks, such as the necessity to either package all the segments together or to

**TABLE 2** | Divergence of *Nanoviridae* from *Geminiviridae* and *Circoviridae*.

Type	Attributes	<i>Nanoviridae</i>	<i>Geminiviridae</i>	<i>Circoviridae</i>
		<b>Nanovirus</b>	<b>Babuvirus</b>	<b>Begomovirus</b>
<b>DNA</b>	Shape	Circular, ssDNA	Circular, ssDNA	Circular, ssDNA
	Partite	Multipartite (8–11 segments)	Multipartite (6 segments)	Monopartite or Bipartite
	ORFs	1 ORF in each component	1 or 2 ORFs in components	6–7 in DNA A 2 in DNA B
	Stem loop (5' - 3')	TANTATTAC	TATTATTAC	TATTATTAC
	Satellite molecules	~3–4	1	2 (1 alpha and 1 Beta)
<b>Size</b>	Length	~1 kb	~1–1.1 kb	~2.5–3 kb
	Diameter	18–19 nm	17–20 nm	18–20 nm
<b>Transmission</b>	Vector	Aphids	Aphids	Whitefly, Leafhopper, Tree-hopper, Aphid
	Tissue tropism	Phloem	Phloem	Phloem, Mesophyll
<b>Infection</b>	Host Range	Plants families: <i>Fabaceae</i> , <i>Caricaceae</i> , <i>Solanaceae</i>	Plants family: <i>Musaceae</i> , <i>Zingiberaceae</i>	Plants families: <i>Fabaceae</i> , <i>Caricaceae</i> , <i>Solanaceae</i> , <i>Convolvulaceae</i>
	Symptoms	Yellows, stunting, mosaic, leaf rolling	Streak mosaic, bushy appearance	Chlorosis, stunting, curling, leaf curling, mottling, leaf distortion
				<b>Circovirus</b>
				Circular, ssDNA
				Monopartite
				2 ORFs in opposite direction
				NANTATTAC
				0
				~1.7–2.1 kb
				~20 nm
				Fecal, oral
				Thymocytes, erythroblastoid cells, embryonal tissues
				Animal families: Birds, pigs, freshwater fish Dogs and humans
				Enlarged lymph nodes, difficulty in breathing, diarrhea, pale skin, jaundice

ensure the co-entry of an ensemble of virus particles containing at least one copy of each genomic segment, (Chao, 1988; Escriu et al., 2007; Ojosnegros et al., 2011). Moreover, there are serious challenges regarding certain features of these viruses that should be fully elucidated, such as replication, genetic expression, genome encapsidation, method of localization within host cells, transport system (i.e., within-host cell-to-cell or long-distance spread), transmission patterns from one host to another by insect vectors, and evolution of multipartite viruses. Recently efforts were made to understand the intriguing multicellular way of life nanoviruses, but it has been demonstrated thus far only one species of the genus *Nanovirus* i.e., FBNSV, not all species of the family *Nanoviridae*.

## GENE EXPRESSION AND VIRAL INFECTION

Multipartite viruses have a set of 8–10 nucleic acid segments, each encapsidated separately. Each segment has a specific number in the host cell to ensure infection. Some viral genes accumulate at low frequency, whereas others dominate at a high frequency (Sicard et al., 2013). This copy number variation in specific genes within individual cells may considerably affect gene expression in most of the organisms (Stranger et al., 2007; Hastings et al., 2009). Each ssDNA segment accumulates in a reproducible manner with a specific relative copy number in a specific host. These copy numbers, each associated with a specific segment, are defined as the “genome formula” and have proved to be specific to the host plant species. The genome formulae in two different host species i.e., *Vicia faba* and *Medicago truncatula* showed clear variations in the relative frequencies of the eight FBNSV segments calculated in within-host viral populations (Sicard et al., 2013). Although the discovery of the genome formula is remarkable in the biology of multipartite viruses, certain gaps need to be addressed; whether the genome formula is also controlled in the same manner as that in other multipartite viruses, whether it has a role in genetic and phenotypic expression, and whether it is an adaptive and evolvable trait.

The mechanism by which multipartite viruses manage to efficiently infect individual cells with all their segments with whole-genome information is a long-standing mystery. Initially, two possibilities were considered: (i) the particles could penetrate the cells massively with any probability independent of the identity of the contained segment, and (ii) multipartite viruses could somehow sort the particles that enter a cell depending on the encapsidated segment and promote the selective entry of the complete set of the viral genetic information (Sicard et al., 2013, 2016; Dall’ara et al., 2016). This mystery was solved by localizing and quantifying the genome segments of a nanovirus in host plant tissues. It was identified that the segments rarely co-occurred within individual cells; instead, distinct segments accumulated independently in different cells, and that the viral system was functional through complementation across cells (Sicard et al., 2016). These findings deviate from the classical conceptual framework in virology and suggest that various viral particles can localize themselves in separate neighboring cells to

produce infection at a multicellular tissue level, thus revealing that the collective presence of all viral genomes in a particular cell is not the basis for infectivity (Sicard et al., 2019). However, these findings are limited to only one nanovirus species i.e., FBNSV. Whether all nanovirus behave like FBNSV is still a question yet to be answered.

## SHORT AND LONG DISTANCE MOVEMENT

Generally, monopartite viruses transfer their genome information either when moving from cell-to-cell or across long distances to systemically colonize their host. In contrast, multipartite viruses bundle their genetic information in separate virus particles, which must somehow come together to cause infection, as viral trafficking within the host plant is multifarious (Hipper et al., 2013). Three models of movements within the host have been suggested in both monopartite and multipartite viruses. Some plant virus species demonstrate both cell-to-cell movement and movement across long distances as mature virus particles. Some can move from cell-to-cell as nucleoprotein complexes (Lazarowitz and Beachy, 1999) but are not capable of long-distance movement because of their inability to assemble into mature virus particles. Finally, some viral species can spread both by cell-to-cell movement and movement in the plant vasculature as nucleoprotein complexes even without containing the protein coat (Carluccio and Stavolone, 2014). There exists a considerable gap in the literature on the differentiation between multipartite virus movement mechanisms, including those of *Nanoviridae* members and other viruses, however, for multipartite viruses, it is predicted that the multiplicity of cellular infection (MOI) should reach very high values (up to hundreds) for the maintenance of genome integrity (Iranzo and Manrubia, 2012). Owing to the lack of data regarding the movement of multipartite viruses, more investigation is needed with focus on species with multiple segments.

## VECTOR TRANSMISSION

Transmission of viruses, either monopartite or multipartite, mostly requires a particular insect vector (Goodman, 1977; Hogenhout et al., 2008; Hull, 2014; Sicard et al., 2015). In the case of *Nanoviridae* members, aphids transmit nanoviruses and babuviruses (Vetten et al., 2005; Almeida et al., 2009; Sicard et al., 2015), while *Myndus tiffany*, a planthopper, transmits CFDV and is considered a major factor in categorizing CFDV as a separate, unassigned species (Gronenborn et al., 2018). No reports regarding the transmission of *Nanoviridae* members either mechanically or through seeds exist to date because of their restriction to the phloem of infected host plants (Grigoras et al., 2018). Babuviruses are transmitted through finite aphid vectors *Pentalonia nigronervosa* and *Micromyzus kalimpongensis* (Almeida et al., 2009; Bressan and Watanabe, 2011; Ghosh et al., 2015; Halbert and Baker, 2015; Qazi, 2016; **Figure 4E**). In contrast, nanoviruses can be transmitted by various aphid

species, in particular *Aphis craccivora*, *A. gossypii*, *Acyrtosiphon pisum*, *Myzus persicae*, and *Macrosiphum euphorbiae* are the most effective vector species for nanoviruses (Sano et al., 1998; Sicard et al., 2015; Gaafar et al., 2016; Richet et al., 2019; **Figure 4F**). Among these aphid species, *A. craccivora* is the most abundant and efficient vector, which transmits MDV, SCSV, FBNYV and FBNSV from plant to plant (Franz et al., 1998; Sicard et al., 2015; Gallet et al., 2018; Webster et al., 2018). Some nanoviruses are transmitted by more than one aphid species, but transmission efficiency varies accordingly, e.g., *A. craccivora* transmits SCSV more efficiently than *M. persicae* (Franz et al., 1998; Sicard et al., 2015). In this way, these nanoviruses can have far-reaching effects outside their pivoting areas.

## TRANSLOCATION OF NANOVIRUSES WITHIN APHID VECTORS

Similar to luteoviruses and geminiviruses, nanoviruses are transmitted in a circular non-propagative manner in their insect vectors (Hogenhout et al., 2008). Virus particles acquired from the infected plant need to cross from the aphids' gut into the hemolymph; within the hemolymph they are transported to the salivary glands (Blanc et al., 2014), followed by injection into new plants during probing. To ensure successful passage of the integral genome to a new host plant, especially in the case of multipartite viruses such as nanoviruses, it is assumed that at least one functional particle of each type must be transmitted (Iranzo and Manrubia, 2012). Several factors e.g., the accumulation of distinct genome segments at different frequencies (Sicard et al., 2013; Sánchez-Navarro et al., 2013), the stability variations within the host plants (Vaughan et al., 2014), along with the degradation and the relative frequency changes in the segments during the passage within the insect vectors (Sicard et al., 2015) and the impacts of transmission-related bottlenecks (Gallet et al., 2018) may result in the loss of genetic information. It was a highly contentious issue that how the most labile particles can be transmitted as efficiently as the others.

Franz et al. (1999) proposed an aphid helping factor to facilitate virus transport which was confirmed and recognized as NSP (Grigoras et al., 2018). Recently, its potential function investigated as distinct proteins and genome segments of the nanovirus FBNSV were remarkably monitored during transcytosis through the gut and salivary gland cells of its aphid vector *Acyrtosiphon pisum* using a combination of fluorescence *in situ* hybridization and immunofluorescence (Di Mattia et al., 2020). FBNSV follows a route similar to that of the geminiviruses but distinct from that of the luteoviruses, as demonstrated by transportation through cells of the anterior midgut and principal salivary gland. A large number of virus particles enter each susceptible cell to keep distinct genome segments together (Di Mattia et al., 2020; Gaafar and Ziebell, 2020). Previously, similar studies were conducted to track the BBTV (genus *Babuvirus*) within its aphid vector by monitoring the coat protein (Bressan and Watanabe, 2011; Watanabe and Bressan, 2013; Watanabe et al., 2016).

## ROLE OF NUCLEAR SHUTTLE PROTEIN IN THE TRANSMISSION

Vector transmission of nanoviruses requires a viral factor or a helper component in addition to the virus particles (Franz et al., 1999). DNA N is the most variable genome component among all components of nanovirus (FBNSV) (Grigoras et al., 2010b). The FBNSV was reconstituted successfully as a fully infectious and sustainably insect-transmissible nanovirus from its multiple cloned DNAs (Grigoras et al., 2009). Recently, the preclusion of aphid transmission was observed when the agroinfectious clones of all segments of FBNSV, except the segment N, were inoculated in a plant though this plant showed similar symptoms as plants that were inoculated with all the eight viral components (Grigoras et al., 2018). The virions that were produced within the plants inoculated with the seven components, excluding DNA-N, abolished the aphid transmission as well when a mutated NSP with a 13-amino acid tag at the carboxyl-terminus was introduced but restored aphid transmission with the introduction of DNA-N of another nanovirus PNYDV (Grigoras et al., 2018), which reinforced the mandatory role of NSP in viral accumulation into the gut cells of the aphid. Co-localization of NSP and coat protein with other viral genome segments, suggest that NSP-virus particle complexes are the viral form that cycles within the aphid body (Di Mattia et al., 2020; Gaafar and Ziebell, 2020). Vector transmission is the major and best-documented mode of transmission of plant viruses, but many gaps i.e., purpose of self interactions between NSPs during translocation, impact of the changes in virus formulas on virus transmission etc., need to be addressed.

## PHYLOGENETIC ANALYSIS OF CP AND REP

Little has been known about the variability and molecular evolution of nanoviruses. The nucleotide substitution rate of  $1.78 \times 10^{-3}$  substitutions per nucleotide per year was observed in FBNSV (Grigoras et al., 2010b) whereas  $1.4 \times 10^{-4}$  substitutions per nucleotide per year was determined in local evolution of BBTV in Hawaii (based only on a single base change) (Almeida et al., 2009). Phylogenetic relationships and pairwise sequence identity calculations also depict the linkages and variations among various types of viruses in a better way (Howarth and Vandemark, 1989). In our study, phylogenetic analysis of DNA R (Rep protein) and DNA S (coat protein) was conducted using different nanovirus and babuvirus species. Master Rep is the most similar segment in nanoviruses as well as in babuviruses and is responsible for replication while CP plays a key role in many steps of the infection cycle i.e., translation, targeting of the viral genome to its site of replication, cell-to-cell and/or systemic movement of the virus, symptomatology and virulence of the infection etc., to ensure viral infection (Bol, 2008). Phylogenetic relationships were analyzed using the iTOL (Letunic and Bork, 2019). The Nevic file for iTOL was generated using the MEGA7 program where the multiple sequence alignment tool MUSCLE used to align all sequences. Genome segments of about 60

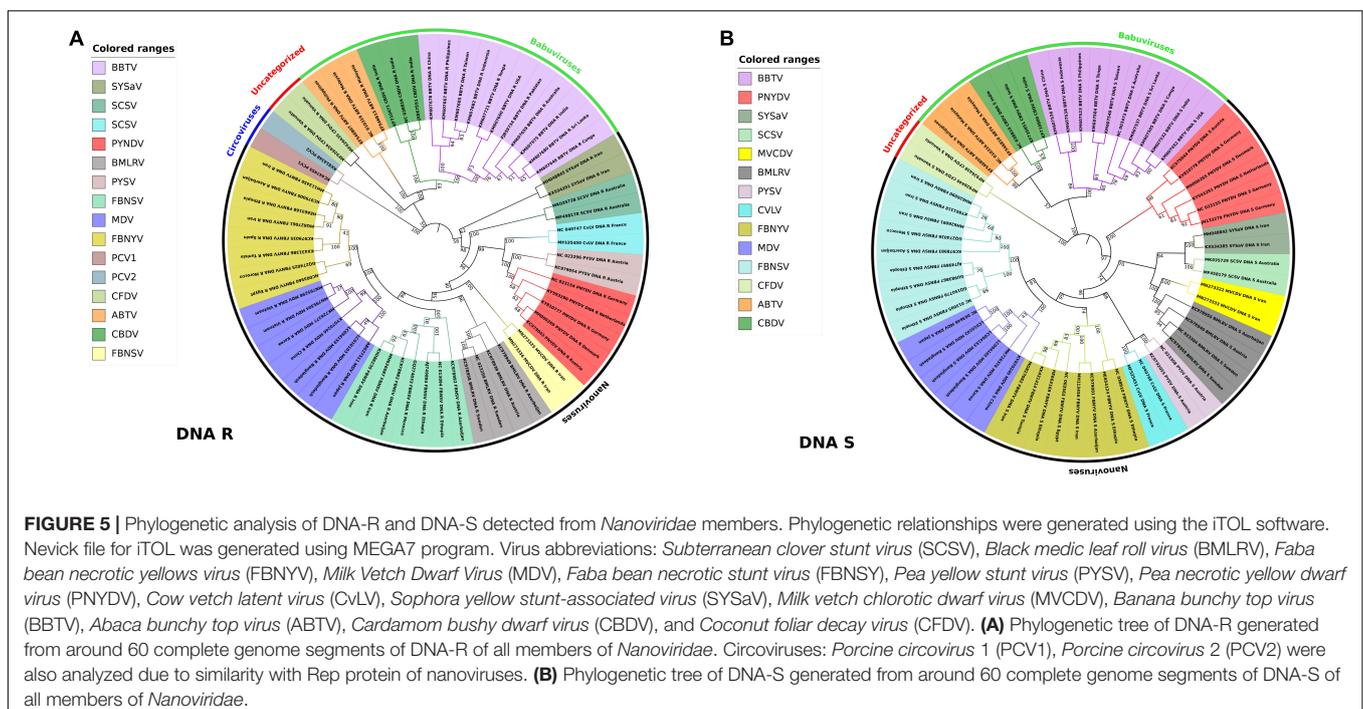
reported species i.e., both nanoviruses and babuviruses were analyzed. In case of DNA R, circoviruses were taken into consideration as well owing to their similarity with the Rep protein of nanoviruses (Gibbs and Weiller, 1999; **Figures 5A,B**). Phylogenetic analysis reveals that each species members share same clade in both cases i.e., DNA R and DNA S due to high similarity within the species members than other species members. As, the isolates of each virus were grouped among virus species, and it was confirmed that nanoviruses and babuviruses were also clearly distinguished.

## NANOVIRIDAE AS AN ECONOMIC THREAT

Agriculture is assumed to be one of the sectors most vulnerable to plant viruses, owing to the potential of viruses to affect plants on a significant scale. Agriculture is considered a fundamental pillar in the world economy and society at large, as it remains a key sector in food supply and is a major source of income. According to the Food and Agriculture Organization of the United Nations, estimated crop losses due to pathogens including plant viruses have been reported to be 20–40% at national and regional levels. Severe damage by plant viruses as well as other pests can significantly decrease the yield of crops as well as their quality, resulting in compromised financial returns due to less produce and lower quality. Plant virus outbreaks, specifically those due to *Geminiviridae* members, have already proven to be a huge setback to the economy in different countries. However, the substantial and devastating consequences of emerging *Nanoviridae* members on crops (Johnstone and Mclean, 1987; Hull, 2014), and the associated economic and social impacts, have largely been

underestimated in the agricultural world. In the early 2000s, a sporadic outbreak of FBNSYV was reported in Spain (Ortiz et al., 2006). In 2016, a survey of 33 symptomatic faba bean sites in central Germany was conducted toward the end of the growing season to analyze the suspected virus spectrum, and PNYDV was found as the major causal agent in all the sites. A close relationship was observed between PNYDV abundance, symptom-intensity, and a corresponding yield decline in grain weight and crude protein. Combining the relative yield level for each symptom category with its respective appearance, the overall yield gap at the field scale was extrapolated to 4.1 and 9.2% for grain yield and 3.9 and 1.2% for crude protein (Saucke et al., 2019). Furthermore, in the epidemic that occurred in central Germany in 2016, the focal appearance with a blackish core can be regarded as a PNYDV-specific feature for temperate faba beans (Ziebell, 2017; Saucke et al., 2019).

Considering the importance of host species infected by babuviruses and nanoviruses categorically may help in the development of broad-scale adversary agents. Bananas are among the top 10 food plants, especially valued in the tropics and are the source of staple food, nutrition and income for millions of banana farmers worldwide. These factors have led to its high demand and the ultimate increase in production over the last decade. BBTV outbreaks alone across 2007–2010 in different states of India caused production losses of at least US\$50 million (Balasubramanian and Selvarajan, 2014). *Vicia faba*, a bean family member found in the Middle East, the Mediterranean region, China, and Ethiopia, is a multipurpose crop used for both food and fodder (hay, silage, and straw). More than 50 species of the bean family are used in human food production as seeds, as an important livestock feed, and for economic benefit owing to their value and consideration as a cash crop in Egypt and



Sudan. Along with bean family members, recent developments clearly outline the expansion in the host range of nanoviruses (as mentioned above). Among these new hosts, papaya, tomato and pepper are the most important ones (though parsley is a valuable member as well). Papaya (*C. papaya* L.) is the third most cultivated fruit in the world and found in both tropical and subtropical zones. It is produced in about 60 countries, mainly in developing countries, with an estimated production of 11.22 Mt (annual growth rate 4.35 percent between 2002 and 2010). The high nutritional and medicinal content of papaya make it an attractive crop for farmers to grow. Tomato (*S. lycopersicum*) is an extremely important Solanaceae member along with pepper (*C. annuum*); both are produced and consumed by people across the world and are used in many cuisines worldwide. In 2017, the worldwide production of tomatoes totaled 170.8 million tons, while pepper production was 576,949 tons in 2018. These high numbers of production are directly and indirectly connected with food security and economic growth in the countries that produce them. Thus, one can speculate that nanoviruses might have the ability for significant impact on these newly reported food crops by affecting their yield (Grigoras et al., 2008; Rosario et al., 2012; Lal et al., 2018, 2020). So, expanding host range threatens to develop into unexpected and serious epidemics but this prediction is still somewhat obscure.

## FUTURE CHALLENGES REGARDING NANOVIRIDAE

Recent developments highlight the diversity in host ranges of the *Nanoviridae* members, with strong evidence indicating that

an increasing number of host species will be reported with time. Along with *Geminiviridae*, *Nanoviridae* members also play a notable role in the plant world, which necessitates equal attention to geminiviruses in understanding their complexities. Particularly, their mode of infection, method of localization, evolutionary history, host ranges, multicellular way of life, preferred hosts and environment, and transmission pattern are all features which remain to be investigated in detail. The agriculture sector has a new emerging threat of *Nanoviridae* infection. It is the collective responsibility of the scientific community to develop a thorough plan and policy to counteract this before a devastating effect on food security and the global economy is realized.

## AUTHOR CONTRIBUTIONS

AL, E-JK, and SL outlined and conceptualized the review theme. AL wrote the first draft of the manuscript. J-KK, TV, IS, PH, E-JK, and SL contributed to manuscript preparation and revision, and also read and approved the submitted version. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the National Research Foundation of Korea grant funded by the Korean government (MSIT) (NRF-2020R1A2C2009946).

## REFERENCES

- Abbas, A. A., Taylor, L. J., Dothard, M. I., Leiby, J. S., Fitzgerald, A. S., Khatib, L. A., et al. (2019). Redondoviridae, a family of small, circular DNA viruses of the human oro-respiratory tract associated with periodontitis and critical illness. *Cell Host Microbe* 25, 719–729. doi: 10.1016/j.chom.2019.05.014
- Abraham, A. D., Bencharki, B., Torok, V., Katul, L., Varrelmann, M., and Vetten, H. J. (2010). Two distinct nanovirus species infecting faba bean in Morocco. *Arch. Virol.* 155, 37–46. doi: 10.1007/s00705-009-0548-9
- Abraham, A. D., Varrelmann, M., and Josef Vetten, H. (2012). Three distinct nanoviruses, one of which represents a new species, infect faba bean in Ethiopia. *Plant Dis.* 96, 1045–1053. doi: 10.1094/pdis-09-11-0734-re
- Almeida, R. P., Bennett, G. M., Anhalt, M. D., Tsai, C.-W., and O'grady, P. (2009). Spread of an introduced vector-borne banana virus in Hawaii. *Mol. Ecol.* 18, 136–146. doi: 10.1111/j.1365-294x.2008.04009.x
- Amin, I., Qazi, J., Mansoor, S., Ilyas, M., and Briddon, R. W. (2008). Molecular characterisation of banana bunchy top virus (BBTV) from Pakistan. *Virus Genes* 36, 191–198. doi: 10.1007/s11262-007-0168-y
- Aronson, M. N., Meyer, A. D., Györgyey, J., Katul, L., Vetten, H. J., Gronenborn, B., et al. (2000). Clink, a nanovirus-encoded protein, binds both pRB and SKP1. *J. Virol.* 74, 2967–2972. doi: 10.1128/jvi.74.7.2967-2972.2000
- Balasubramanian, V., and Selvarajan, R. (2014). Genetic diversity and recombination analysis in the coat protein gene of banana bract mosaic virus. *Virus Genes* 48, 509–517. doi: 10.1007/s11262-014-1056-x
- Beetham, P. R., Hafner, G. J., Harding, R. M., and Dale, J. L. (1997). Two mRNAs are transcribed from banana bunchy top virus DNA-1. *J. Gen. Virol.* 78, 229–236. doi: 10.1099/0022-1317-78-1-229
- Biagini, P., De Micco, P., and De Lamballerie, X. (2006). Identification of a third member of the Anellovirus genus ("small anellovirus") in French blood donors. *Arch. Virol.* 151, 405–408. doi: 10.1007/s00705-005-0660-4
- Blanc, S., Drucker, M., and Uzest, M. (2014). Localizing viruses in their insect vectors. *Annu. Rev. Phytopathol.* 52, 403–425. doi: 10.1146/annurev-phyto-102313-045920
- Blatter, J. A., Sweet, S. C., Conrad, C., Danziger-Isakov, L. A., Faro, A., Goldfarb, S. B., et al. (2018). Anellovirus loads are associated with outcomes in pediatric lung transplantation. *Pediatr. Transplant* 22
- Blomme, G., Pløetzer, R., Jones, D., De Langhe, E., Price, N., Gold, C., et al. (2013). A historical overview of the appearance and spread of *Musa* pests and pathogens on the African continent: highlighting the importance of clean *Musa* planting materials and quarantine measures. *Ann. Appl. Biol.* 162, 4–26. doi: 10.1111/aab.12002
- Boevink, P., Chu, P. W. G., and Keese, P. (1995). Sequence of subterranean clover stunt virus DNA: affinities with the geminiviruses. *J. Virol.* 207, 354–361. doi: 10.1006/viro.1995.1094
- Bol, J. F. (2008). Role of capsid proteins. *Methods Mol. Biol.* 451, 21–31. doi: 10.1007/978-1-59745-102-4\_2
- Bressan, A., and Watanabe, S. (2011). Immunofluorescence localisation of banana bunchy top virus (family *Nanoviridae*) within the aphid vector, *Pentalonia nigronervosa*, suggests a virus tropism distinct from aphid-transmitted luteoviruses. *Virus Res.* 155, 520–525. doi: 10.1016/j.virusres.2010.12.005
- Briddon, R. W., Martin, D. P., Roumagnac, P., Navas-Castillo, J., Fiallo-Olivé, E., Moriones, E., et al. (2018). *Alphasatellitidae*: a new family with two subfamilies for the classification of geminivirus- and nanovirus-associated alphasatellites. *Arch. Virol.* 163, 2587–2600. doi: 10.1007/s00705-018-3854-2
- Buchmann, R. C., Asad, S., Wolf, J. N., Mohannath, G., and Bisaro, D. M. (2009). Geminivirus AL2 and L2 proteins suppress transcriptional gene silencing and cause genome-wide reductions in cytosine methylation. *J. Virol.* 83, 5005–5013. doi: 10.1128/jvi.01771-08

- Burns, T. M., Harding, R. M., and Dale, J. L. (1995). The genome organization of banana bunchy top virus: analysis of six ssDNA components. *J. Gen. Virol.* 76, 1471–1482. doi: 10.1099/0022-1317-76-6-1471
- Carluccio, A. V., and Stavelone, L. (2014). Interference of Brefeldin A in viral movement protein tubules assembly. *Plant Signal. Behav.* 9:e29121. doi: 10.4161/psb.29121
- Chao, L. (1988). Evolution of sex in RNA viruses. *J. Theor. Biol.* 133, 99–112. doi: 10.1016/s0022-5193(88)80027-4
- Chao, L. (1991). Levels of selection, evolution of sex in RNA viruses, and the origin of life. *J. Theor. Biol.* 153, 229–246. doi: 10.1016/s0022-5193(05)80424-2
- Cheung, A. K. (2006). Rolling-circle replication of an animal circovirus genome in a theta-replicating bacterial plasmid in *Escherichia coli*. *J. Virol.* 80, 8686–8694. doi: 10.1128/jvi.00655-06
- Dall'ara, M., Ratti, C., Bouzoubaa, S. E., and Gilmer, D. (2016). Ins and outs of multipartite positive-strand RNA plant viruses: packaging versus systemic spread. *Viruses* 8:228. doi: 10.3390/v8080228
- Dayaram, A., Galatowitsch, M., Harding, J. S., Argüello-Astorga, G. R., and Varsani, A. (2014). Novel circular DNA viruses identified in *Procardulia grayi* and *Xanthocnemis zealandica* larvae using metagenomic approaches. *Infect. Genet. Evol.* 22, 134–141. doi: 10.1016/j.meegid.2014.01.013
- Di Mattia, J., Vernerey, M. S., Yvon, M., Pirolles, E., Villegas, M., Gaafar, Y., et al. (2020). Route of a multipartite nanovirus across the body of its aphid vector. *J. Virol.* 94:e01998-19.
- Escriu, F., Fraile, A., and García-Arenal, F. (2007). Constraints to genetic exchange support gene coadaptation in a tripartite RNA virus. *PLoS Pathog.* 3:e8. doi: 10.1371/journal.ppat.0030008
- Franz, A., Makkouk, K. M., and Vetten, H. J. (1997). Host range of faba bean necrotic yellows virus and potential yield loss in infected faba bean. *Phytopathol. Mediterr.* 36, 94–103.
- Franz, A., Makkouk, K. M., and Vetten, H. J. (1998). Acquisition, retention and transmission of faba bean necrotic yellows virus by two of its aphid vectors, *Aphis craccivora* (Koch) and *Acyrtosiphon pisum* (Harris). *Phytopathology* 146, 347–355. doi: 10.1111/j.1439-0434.1998.tb04703.x
- Franz, A. W. E., Van Der Wilk, F., Verbeek, M., Dulleman, A. M., and Van Den Heuvel, J. F. J. M. (1999). Faba bean necrotic yellows virus (Genus *Nanovirus*) requires a helper factor for its aphid transmission. *J. Virol.* 262, 210–219. doi: 10.1006/viro.1999.9904
- Gaafar, Y., Cordsen Nielsen, G., and Ziebell, H. (2018). Molecular characterisation of the first occurrence of pea necrotic yellow dwarf virus in Denmark. *New Dis. Rep.* 37, 16–16. doi: 10.5197/j.2044-0588.2018.037.016
- Gaafar, Y., Grausgruber-Gröger, S., and Ziebell, H. (2016). *Vicia Faba*, *V. Sativa* and *Lens Culinaris* as new hosts for pea necrotic yellow dwarf virus in Germany and Austria. *New Dis. Rep.* 34:28. doi: 10.5197/j.2044-0588.2016.034.028
- Gaafar, Y., Timchenko, T., and Ziebell, H. (2017). First report of pea necrotic yellow dwarf virus in the Netherlands. *New Dis. Rep.* 35:23. doi: 10.5197/j.2044-0588.2017.035.023
- Gaafar, Y. Z. A., and Ziebell, H. (2020). Aphid transmission of nanoviruses. *Arch. Insect Biochem. Physiol.* 104:e21668.
- Gallet, R., Fabre, F., Thébaud, G., Sofonea, M. T., Sicard, A., Blanc, S., et al. (2018). Small bottleneck size in a highly multipartite virus during a complete infection cycle. *J. Virol.* 92:e00139-18.
- Ghosh, A., Das, A., Vijayanandraj, S., and Mandal, B. (2015). Cardamom bushy dwarf virus infection in large cardamom alters plant selection preference, life stages, and fecundity of aphid vector, *Micromyzus kalimpongensis* (Hemiptera: Aphididae). *Environ. Entomol.* 45, 178–184. doi: 10.1093/ee/nvv161
- Gibbs, M. J., and Weiller, G. F. (1999). Evidence that a plant virus switched hosts to infect a vertebrate and then recombined with a vertebrate-infecting virus. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8022–8027. doi: 10.1073/pnas.96.14.8022
- Goodman, R. M. (1977). Single-stranded DNA genome in a whitefly-transmitted plant virus. *J. Virol.* 83, 171–179. doi: 10.1016/0042-6822(77)90220-3
- Grigoras, I., Del Cueto Ginzo, A. I., Martin, D. P., Varsani, A., Romero, J., Mammadov, A. C., et al. (2014). Genome diversity and evidence of recombination and reassortment in nanoviruses from Europe. *J. Gen. Virol.* 95, 1178–1191. doi: 10.1099/vir.0.063115-0
- Grigoras, I., Gronenborn, B., and Vetten, H. (2010a). First report of a nanovirus disease of pea in Germany. *Plant Dis.* 94, 642–642. doi: 10.1094/pdis-94-5-0642c
- Grigoras, I., Timchenko, T., Grande-Pérez, A., Katul, L., Vetten, H.-J., and Gronenborn, B. (2010b). High variability and rapid evolution of a nanovirus. *J. Virol.* 84:9105. doi: 10.1128/jvi.00607-10
- Grigoras, I., Timchenko, T., and Gronenborn, B. (2008). Transcripts encoding the nanovirus master replication initiator proteins are terminally redundant. *J. Gen. Virol.* 89, 583–593. doi: 10.1099/vir.0.83352-0
- Grigoras, I., Timchenko, T., Katul, L., Grande-Pérez, A., Vetten, H.-J., and Gronenborn, B. (2009). Reconstitution of authentic nanovirus from multiple cloned DNAs. *J. Virol.* 83, 10778–10787. doi: 10.1128/jvi.01212-09
- Grigoras, I., Vetten, H.-J., Commandeur, U., Ziebell, H., Gronenborn, B., and Timchenko, T. (2018). Nanovirus DNA-N encodes a protein mandatory for aphid transmission. *J. Virol.* 522, 281–291. doi: 10.1016/j.virol.2018.07.001
- Gronenborn, B. (2004). Nanoviruses: genome organisation and protein function. *Vet. Microbiol.* 98, 103–109. doi: 10.1016/j.vetmic.2003.10.015
- Gronenborn, B., Randles, J. W., Knierim, D., Barrière, Q., Vetten, H. J., Warthmann, N., et al. (2018). Analysis of DNAs associated with coconut foliar decay disease implicates a unique single-stranded DNA virus representing a new taxon. *Sci. Rep.* 8:5698.
- Halbert, S. E., and Baker, C. A. (2015). *Banana bunchy top virus* and its vector *Pentalonia nigronervosa* (Hemiptera: Aphididae). *Pathol. Circ.* 47:8.
- Harrison, B. D. (1985). Advances in geminivirus research. *Annu. Rev. Phytopathol.* 23, 55–82. doi: 10.1146/annurev.py.23.090185.000415
- Hassan-Sheikhi, P., Heydarnejad, J., Massumi, H., Kraberger, S., and Varsani, A. (2020). Novel nanovirus and associated alphasatellites identified in milk vetch plants with chlorotic dwarf disease in Iran. *Virus Res.* 276:197830. doi: 10.1016/j.virusres.2019.197830
- Hastings, P. J., Lupski, J. R., Rosenberg, S. M., and Ira, G. (2009). Mechanisms of change in gene copy number. *Nat. Rev. Genet.* 10, 551–564. doi: 10.1038/nrg2593
- Hayakawa, T., Kojima, K., Nonaka, K., Nakagaki, M., Sahara, K., Asano, S.-I., et al. (2000). Analysis of proteins encoded in the bipartite genome of a new type of parvo-like virus isolated from silkworm — structural protein with DNA polymerase motif. *Virus Res.* 66, 101–108. doi: 10.1016/s0168-1702(99)00129-x
- Heydarnejad, J., Kamali, M., Massumi, H., Kvarnheden, A., Male, M. F., Kraberger, S., et al. (2017). Identification of a nanovirus-alphasatellite complex in *Sophora alopecuroides*. *Virus Res.* 235, 24–32. doi: 10.1016/j.virusres.2017.03.023
- Hipper, C., Brault, V., Ziegler-Graff, V., and Revers, F. (2013). Viral and cellular factors involved in phloem transport of plant viruses. *Front. Plant Sci.* 4:154. doi: 10.3389/fpls.2013.00154
- Hogenhout, S. A., Ammar, E.-D., Whitfield, A. E., and Redinbaugh, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annu. Rev. Phytopathol.* 46, 327–359. doi: 10.1146/annurev.phyto.022508.092135
- Horsler, C. L., Harding, R. M., and Dale, J. L. (2001). *Banana bunchy top nanovirus* DNA-1 encodes the ‘master’ replication initiation protein. *J. Gen. Virol.* 82, 459–464. doi: 10.1099/0022-1317-82-2-459
- Howarth, A. J., and Vandemark, G. J. (1989). Phylogeny of geminiviruses. *J. Gen. Virol.* 70, 2717–2727. doi: 10.1099/0022-1317-70-10-2717
- Hull, R. (2014). *Plant Virology*. Cambridge, MA: Academic press.
- Iranzo, J., and Manrubia, S. C. (2012). Evolutionary dynamics of genome segmentation in multipartite viruses. *Proc. R. Soc. B.* 279, 3812–3819. doi: 10.1098/rspb.2012.1086
- Jeske, H. (2009). “Geminiviruses,” in *TT Viruses: The Still Elusive Human Pathogens*, eds E.-M. De Villiers, and H. Z. Hausen (Berlin: Springer Berlin Heidelberg), 185–226.
- Jeske, H. (2018). Barcoding of plant viruses with circular single-stranded DNA based on rolling circle amplification. *Viruses* 10:469. doi: 10.3390/v10090469
- Johnstone, G. R., and Mclean, G. D. (1987). Virus diseases of subterranean clover. *Ann. Appl. Biol.* 110, 421–440.
- Katul, L., Timchenko, T., Gronenborn, B., and Vetten, H. J. (1998). Ten distinct circular ssDNA components, four of which encode putative replication-associated proteins, are associated with the faba bean necrotic yellows virus genome. *J. Gen. Virol.* 79, 3101–3109. doi: 10.1099/0022-1317-79-12-3101
- Kenyon, L., Tsai, W.-S., Shih, S.-L., and Lee, L.-M. (2014). Emergence and diversity of begomoviruses infecting solanaceous crops in East and Southeast Asia. *Virus Res.* 186, 104–113. doi: 10.1016/j.virusres.2013.12.026
- Kil, E.-J., Kim, S., Lee, Y.-J., Byun, H.-S., Park, J., Seo, H., et al. (2016). *Tomato yellow leaf curl virus* (TYLCV-IL): a seed-transmissible geminivirus in tomatoes. *Sci. Rep.* 6:19013.

- Koonin, E. V., Dolja, V. V., and Krupovic, M. (2015). Origins and evolution of viruses of eukaryotes: the ultimate modularity. *J. Virol.* 479, 2–25. doi: 10.1016/j.virol.2015.02.039
- Krupovic, M., Ghabrial, S. A., Jiang, D., and Varsani, A. (2016). Genomoviridae: a new family of widespread single-stranded DNA viruses. *Arch. Virol.* 161, 2633–2643. doi: 10.1007/s00705-016-2943-3
- Krupovic, M., Varsani, A., Kazlauskas, D., Breitbart, M., Delwart, E., Rosario, K., et al. (2020). *Cressdnaviricota*: a virus phylum unifying 7 families of Rep-encoding viruses with single-stranded, circular DNA genomes. *J. Virol.* 94:e00582-20.
- Lal, A., Kil, E.-J., Byun, H.-S., Zarghami, D. S., Kim, J.-K., and Lee, S. (2018). First report of milk vetch dwarf virus associated with dwarfism in papaya in Korea. *Plant Dis.* 102:2666. doi: 10.1094/pdis-01-18-0190-pdn
- Lal, A., Kil, E.-J., Thuy, V. T. B., Fadhila, C., Ho, P. T., Byun, H.-S., et al. (2020). Milk vetch dwarf virus infection in the *Solanaceae* and *Caricaceae* families in Southeast Asia. *Plant Pathol.* 69, 1026–1033. doi: 10.1111/ppa.13196
- Lazarowitz, S. G., and Beachy, R. N. (1999). Viral movement proteins as probes for intracellular and intercellular trafficking in plants. *Plant Cell* 11:535. doi: 10.2307/3870882
- Letunic, I., and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res.* 47, W256–W259.
- Malathi, V. G., and Dasgupta, I. (2019a). Insights into the world of ssDNA viruses. *Virusdisease* 30, 1–2. doi: 10.1007/s13337-019-00529-2
- Malathi, V. G., and Renuka Devi, P. (2019b). ssDNA viruses: key players in global virome. *Virusdisease* 30, 3–12. doi: 10.1007/s13337-019-00519-4
- Mandal, B. (2010). Advances in small isometric multicomponent ssDNA viruses infecting plants. *Indian J. Virol.* 21, 18–30. doi: 10.1007/s13337-010-0010-3
- Mandal, B., Mandal, S., Pun, K. B., and Varma, A. (2004). First report of the association of a nanovirus with foorky disease of large cardamom in India. *Plant Dis.* 88, 428–428. doi: 10.1094/pdis.2004.88.4.428a
- Mansoor, S., Briddon, R. W., Zafar, Y., and Stanley, J. (2003). Geminivirus disease complexes: an emerging threat. *Trends Plant Sci.* 8, 128–134. doi: 10.1016/s1360-1385(03)00007-4
- Merits, A., Fedorkin, O. N., Guo, D., Kalinina, N. O., and Morozov, S. Y. (2000). Activities associated with the putative replication initiation protein of coconut foliar decay virus, a tentative member of the genus *Nanovirus*. *J. Gen. Virol.* 81, 3099–3106. doi: 10.1099/0022-1317-81-12-3099
- Nee, S. (1987). The evolution of multicompartmental genomes in viruses. *J. Mol. Evol.* 25, 277–281.
- Ng, T. F. F., Zhang, W., Sachsenröder, J., Kondov, N. O., Da Costa, A. C., Vega, E., et al. (2015). A diverse group of small circular ssDNA viral genomes in human and non-human primate stools. *Virus Evol.* 1:vev017. doi: 10.1093/ve/vev017
- Niagro, F. D., Forsthoefel, A. N., Lawther, R. P., Kamalanathan, L., Ritchie, B. W., Latimer, K. S., et al. (1998). Beak and feather disease virus and porcine circovirus genomes: intermediates between the geminiviruses and plant circoviruses. *Arch. Virol.* 143, 1723–1744. doi: 10.1007/s007050050412
- Ojosnegros, S., García-Arriaza, J., Escarmis, C., Manrubia, S. C., Perales, C., Arias, A., et al. (2011). Viral genome segmentation can result from a trade-off between genetic content and particle stability. *PLoS Genet.* 7:e1001344. doi: 10.1371/journal.pgen.1001344
- Ortiz, V., Navarro, E., Castro, S., Carazo, G., and Romero, J. (2006). Incidence and transmission of *Faba bean necrotic yellows virus* (FBNYV) in Spain. *Span. J. Agric. Res.* 4, 255–260. doi: 10.5424/sjar/2006043-200
- Pressing, J., and Reaney, D. (1984). Divided genomes and intrinsic noise. *J. Mol. Evol.* 20, 135–146. doi: 10.1007/bf02257374
- Qazi, J. (2016). Banana bunchy top virus and the bunchy top disease. *J. Gen. Plant Pathol.* 82, 2–11. doi: 10.1007/s10327-015-0642-7
- Randles, J., Chu, P., Dale, J., Harding, R., Hu, J., Katul, L., et al. (2000). “Nanoviridae,” in *Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses*, eds M. H. V. van Regenmortel, et al. (London: Elsevier/Academic Press).
- Richet, C., Kraberger, S., Filloux, D., Bernardo, P., Harkins, G. W., Martin, D. P., et al. (2019). Novel circular DNA viruses associated with *Apiaceae* and *Poaceae* from South Africa and New Zealand. *Arch. Virol.* 164, 237–242. doi: 10.1007/s00705-018-4031-3
- Rodrigues, G. B., Rocha Sobrinho, G. G., Mituti, T., Bergamin Filho, A., Amorim, L., Rezende, J. A. M., et al. (2019). Etiology, occurrence and epidemiology of a begomovirus disease in passionflower in the southwest of Bahia. *Sci. Agric.* 76, 337–343. doi: 10.1590/1678-992x-2017-0272
- Rosario, K., Duffy, S., and Breitbart, M. (2012). A field guide to eukaryotic circular single-stranded DNA viruses: insights gained from metagenomics. *Arch. Virol.* 157, 1851–1871. doi: 10.1007/s00705-012-1391-y
- Rosario, K., Schenck, R. O., Harbeitner, R. C., Lawler, S. N., and Breitbart, M. (2015). Novel circular single-stranded DNA viruses identified in marine invertebrates reveal high sequence diversity and consistent predicted intrinsic disorder patterns within putative structural proteins. *Front. Microbiol.* 6:696.
- Sánchez-Navarro, J. A., Zwart, M. P., and Elena, S. F. (2013). Effects of the number of genome segments on primary and systemic infections with a multipartite plant RNA virus. *J. Virol.* 87:10805. doi: 10.1128/jvi.01402-13
- Sano, Y., Wada, M., Hashimoto, Y., Matsumoto, T., and Kojima, M. (1998). Sequences of ten circular ssDNA components associated with the milk vetch dwarf virus genome. *J. Gen. Virol.* 79, 3111–3118. doi: 10.1099/0022-1317-79-12-3111
- Saucke, H., Uteau, D., Brinkmann, K., and Ziebell, H. (2019). Symptomatology and yield impact of pea necrotic yellow dwarf virus (PNYDV) in faba bean (*Vicia faba* L. minor). *Eur. J. Plant Pathol.* 153, 1299–1315. doi: 10.1007/s10658-018-01643-5
- Saunders, K., Bedford, I. D., Yahara, T., and Stanley, J. (2003). The earliest recorded plant virus disease. *Nature* 422:831. doi: 10.1038/422831a
- Sharman, M., Thomas, J., Skabo, S., and Holton, T. (2008). Abaca bunchy top virus, a new member of the genus *Babuvirus* (family *Nanoviridae*). *Arch. Virol.* 153, 135–147. doi: 10.1007/s00705-007-1077-z
- Sicard, A., Michalakakis, Y., Gutiérrez, S., and Blanc, S. (2016). The strange lifestyle of multipartite viruses. *PLoS Pathog.* 12:e1005819. doi: 10.1371/journal.ppat.1005819
- Sicard, A., Piroles, E., Gallet, R., Vernerey, M.-S., Yvon, M., Urbino, C., et al. (2019). A multicellular way of life for a multipartite virus. *eLife* 8:e43599.
- Sicard, A., Yvon, M., Timchenko, T., Gronenborn, B., Michalakakis, Y., Gutierrez, S., et al. (2013). Gene copy number is differentially regulated in a multipartite virus. *Nat. Commun.* 4:2248.
- Sicard, A., Zeddam, J.-L., Yvon, M., Michalakakis, Y., Gutiérrez, S., and Blanc, S. (2015). Circulative nonpropagative aphid transmission of nanoviruses: an oversimplified view. *J. Virol.* 89, 9719–9726. doi: 10.1128/jvi.00780-15
- Simmonds, P., Adams, M. J., Benkő, M., Breitbart, M., Brister, J. R., Carstens, E. B., et al. (2017). Consensus statement: virus taxonomy in the age of metagenomics. *Nat. Rev. Microbiol.* 15, 161–168.
- Spence, N. J., Phiri, N. A., Hughes, S. L., Mwaniki, A., Simons, S., Oduor, G., et al. (2007). Economic impact of turnip mosaic virus, cauliflower mosaic virus and beet mosaic virus in three Kenyan vegetables. *Plant Pathol.* 56, 317–323. doi: 10.1111/j.1365-3059.2006.01498.x
- Stranger, B. E., Forrest, M. S., Dunning, M., Ingle, C. E., Beazley, C., Thorne, N., et al. (2007). Relative impact of nucleotide and copy number variation on gene expression phenotypes. *J. Sci.* 315, 848–853. doi: 10.1126/science.1136678
- Sun, S.-K. (1961). Studies on the bunchy top disease of bananas. *Spec. Publ.* 10, 82–109.
- Timchenko, T., Katul, L., Sano, Y., De Kouchkovsky, F., Vetten, H. J., and Gronenborn, B. (2000). The master rep concept in nanovirus replication: identification of missing genome components and potential for natural genetic reassortment. *J. Virol.* 74, 189–195. doi: 10.1006/viro.2000.0439
- Tischer, I., Miels, W., Wolff, D., Vagt, M., and Griem, W. (1986). Studies on epidemiology and pathogenicity of porcine circovirus. *Arch. Virol.* 91, 271–276. doi: 10.1007/bf01314286
- Vaughan, R., Tragesser, B., Ni, P., Ma, X., Dragnea, B., and Kao, C. C. (2014). The tripartite virions of the brome mosaic virus have distinct physical properties that affect the timing of the infection process. *J. Virol.* 88:6483. doi: 10.1128/jvi.00377-14
- Vetten, H., Chu, P., Dale, J., Harding, R., Hu, J., Katul, L., et al. (2005). *Nanoviridae*. Cambridge, MA: Elsevier-Academic Press.
- Vetten, H. J., Dale, J. L., Grigoras, I., Gronenborn, B., Harding, R., Randles, J. W., et al. (2012). *Family Nanoviridae in virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. Amsterdam: Elsevier.
- Vetten, H. J., Knierim, D., Rakoski, M. S., Menzel, W., Maiss, E., Gronenborn, B., et al. (2019). Identification of a novel nanovirus in parsley. *Arch. Virol.* 164, 1883–1887. doi: 10.1007/s00705-019-04280-3

- Wanitchakorn, R., Hafner, G. J., Harding, R. M., and Dale, J. L. (2000). Functional analysis of proteins encoded by banana bunchy top virus DNA-4 to -6. *Virus Res.* 81, 299–306. doi: 10.1099/0022-1317-81-1-299
- Wanitchakorn, R., Harding, R., and Dale, J. (1997). *Banana bunchy top virus* DNA-3 encodes the viral coat protein. *Arch. Virol.* 142, 1673–1680. doi: 10.1007/s007050050188
- Watanabe, S., Borthakur, D., and Bressan, A. (2016). Localization of banana bunchy top virus and cellular compartments in gut and salivary gland tissues of the aphid vector *Pentalonia nigronervosa*. *Insect Sci.* 23, 591–602. doi: 10.1111/1744-7917.12211
- Watanabe, S., and Bressan, A. (2013). Tropism, compartmentalization and retention of banana bunchy top virus (*Nanoviridae*) in the aphid vector *Pentalonia nigronervosa*. *J. Gen. Virol.* 94, 209–219. doi: 10.1099/vir.0.047308-0
- Webster, C. G., Pichon, E., Van Munster, M., Monsion, B., Deshoux, M., Gargani, D., et al. (2018). Identification of plant virus receptor candidates in the stylets of their aphid vectors. *J. Virol.* 92:e00432-18.
- Yoon, H. S., Price, D. C., Stepanauskas, R., Rajah, V. D., Sieracki, M. E., Wilson, W. H., et al. (2011). Single-cell genomics reveals organismal interactions in uncultivated marine protists. *Science* 332:714. doi: 10.1126/science.1203163
- Zerbini, F. M., Briddon, R. W., Idris, A., Martin, D. P., Moriones, E., Navas-Castillo, J., et al. (2017). ICTV Virus Taxonomy profile: *Geminiviridae*. *J. Gen. Virol.* 98, 131–133.
- Ziebell, H. (2017). Die virussepidemie an leguminosen 2016 eine folge des klimawandels? *J. Kulturpflanzen* 69, 64–68.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Lal, Vo, Sanjaya, Ho, Kim, Kil and Lee. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.