Advances in Small Isometric Multicomponent ssDNA Viruses Infecting Plants

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Abstract Multicomponent ssDNA plant viruses were discovered during 1990s. They are associated with bunchy top, yellowing and dwarfing diseases of several economic plants under family Musaceae, Leguminosae and Zingiberaceae. In the current plant virus taxonomy, these viruses are classified under the family *Nanoviridae* containing two genera, *Nanovirus* and *Babuvirus*. The family *Nanoviridae* was created with five members in 2005 and by 2010, it has expanded with four additional members. The viruses are distributed in the tropical and subtropical regions of Asia, Australia, Europe and Africa. The viruses are not sap or seed transmissible and are naturally transmitted by aphid vector in a persistent manner. The genome is consisted of several circular ssDNAs of about 1 kb each. Up to 12 DNA components have been isolated from the diseased plant. The major viral proteins encoded by these components are replication initiator protein (Rep), coat protein, cell-cycle link protein, movement protein and a nuclear shuttle protein. Each ssDNA contains a single gene and a noncoding region with a stable stem and loop structure. Several Rep encoding components have been reported from each virus, only one of them designated as master Rep has ability to control replication of the other genomic components. Infectivity of the genomic DNAs was demonstrated only for two nanoviruses, *Faba bean necrotic yellows virus* and *Faba bean necrotic stunt virus* (FBNSV). A group of eight ssDNA components of FBNSV were necessary for producing disease and biologically active progeny viruses. So far, infectivity of genomic components of *Babuvirus* has not been demonstrated.

Keywords *Nanovirus* · *Babuvirus* · ssDNA virus · BBTV · ABTV · CBTV · SCSV · FBNYV · FBNSV · PNYDV · MDV · CFDV

Introduction

There were several historical plant diseases for which the causal virus was a puzzle for a long time (Table 1). Bunchy top of banana [51] and abaca [68], stunt or dwarf of subterranean clover [28] and milk vetch [62], and foorkey of large cardamom [66] are examples of such diseases, which were identified as of viral origin largely based on symptoms and transmission by aphid. Involvement of specific viruses with the diseased plants could not be easily established as recognizable virus-like structure was not readily seen in electron microscope. The causal virus of some of these diseases like bunchy top of banana and dwarfing/stunting of milk vetch and subterranean clover was misunderstood as luteovirus [63, 71, 81]. During late 1980s and early 1990s, when virus purification was successful from several of these diseases, association of smallest isometric virus of 17–20 nm was evident [13, 40, 44, 73, 103]. Subsequently, during 1993–1995, genome sequencing and molecular characterisation revealed that these viruses contained multiple components of single stranded circular DNA (ssDNA) each about 1.0 kb in size. Both the virion and genome size being smallest of all the known plant viruses and having no significant sequence similarities with any other plant viruses, they were classified under the genus *Nanovirus* (nano = small), a new class of plant viruses [78]. Unlike other plant viruses, where viral genes are generally clustered together in one or a few genome components; in the nanoviruses, each gene is located in an independent ssDNA component. As a result, genomic information is distributed in at least 6–8 circular ssDNA

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Table 1 Historical milestones of advances in multicomponent ssDNA viruses infecting plants

Crop	Disease	Country	Milestone year				References
			Disease record	Virus observation	Genome sequence	Infectivity of cloned DNAs	
Banana	Bunchy top	Fiji	1879	1990	1993	–	[53, 103, 32]
Abaca	Bunchy top	Philippines	1915	–	2008	–	[68, 87]
Large cardamom	Foorkey	India	1935	2004	2004	–	[66, 60]
Milk vetch	Dwarf	Japan	1953	1992	1998	–	[62, 40, 86]
Subterranean clover	Stunt	Australia	1956	1988	1995	–	[28, 13, 8]
Coconut	Foliar decay	Vanuatu	1979	1989	1990	–	[18, 73, 82]
Faba bean	Necrotic yellows	Syria	1988	1993	1995	2006	[56, 44, 45, 95]
Faba bean	Necrotic stunt	Ethiopia	1999	–	2009	2009	[23, 25]
Pea	Necrotic yellow dwarf	Germany	2009	–	2010	–	[26]

components [98]. *Nanovirus* having some similarities in genome structure was thought to be a relative of geminivirus that was discovered during 1980s and was known to be the only ssDNA virus infecting plants [15]. Geminivirus, however, having unique twin-isometric virion of 18×30 nm and mono or bipartite genome component, was different from nanoviruses. Vertebrate-infecting ssDNA viruses, circoviruses also have similarities with nanoviruses. It appears that circoviruses have evolved diverging from nanoviruses through host-switching event [24].

In the recent years, there is a considerable progress in our understanding of multicomponent ssDNA plant viruses. A large pool of genome sequence data has been accumulated, new members are discovered and taxonomy has been re-structured. Genome organization and protein function of nanoviruses have been reviewed in 2004 [27]. The present review describes the advances on both *Nanovirus* and *Babuvirus* and the diseases caused by them.

Taxonomic Structure

In the 7th report of International Committee on Taxonomy of Viruses (ICTV), multicomponent ssDNA small plant viruses were classified as genus *Nanovirus* [78]. The work on genome analysis revealed that the legume-infecting ssDNA viruses have closer resemblance among themselves, but they differ substantially from those infecting banana. Because of the biological and molecular distinction, the classification of nanoviruses was revised in the 8th report of ICTV and the family, *Nanoviridae* was created with two genera, *Nanovirus* and *Babuvirus* [98]. The genus *Nanovirus*, included three species, *Faba bean necrotic yellows virus* (FBNYV), *Milk vetch dwarf virus* (MDV) and *Subterranean clover stunt virus* (SCSV). Recently, two new nanoviruses, *Faba bean necrotic stunt virus* (FBNSV)

and *Pea necrotic yellow dwarf virus* (PVYDV) have been reported [25, 26]. The genus *Babuvirus* included only, *Banana bunchy top virus* (BBTV). Recently, *Abaca bunchy top virus* (ABTV) and *Cardamom bushy dwarf virus* (CBDV) have been reported as new virus species under *Babuvirus* [60, 84, 87]. There is only one unassigned species, *Coconut foliar decay virus* (CFDV). At present, a total of nine different viruses are known under the family *Nanoviridae* (Table 2). The guidelines for demarcation of species within the genus have been prescribed by ICTV. The criteria for defining species are <75% identity in overall nucleotide sequence, differential serological reaction, >15% difference in coat protein sequence and differential natural host.

Geographical Distribution

Both nanoviruses and babuviruses are distributed in tropical and subtropical regions of Asia, Australia, Europe and Africa (Fig. 1). BBTV is widely distributed in banana growing countries in the Asia, Pacific region (Australia, China, Guam, India, Pakistan, Sri Lanka, Hawaii, Indonesia, Japan, the Philippines, Taiwan, Tonga, Samoa and Vietnam) and in a few countries in Africa (Egypt, Gabon, Angola and Burundi) [2, 16, 50, 89, 99]. There are many other banana growing regions, South and Central America, the Caribbean, Papua New Guinea, Thailand and in many countries in African and Asia, where BBTV has not been confirmed [43, 89]. ABTV is distributed in Abaca growing regions of the Philippines [87]. CBDV affecting large cardamom is distributed in eastern sub-Himalayan mountains of India [60]. SCSV and MDV occur in Australia and Japan, respectively. Recently, MDV has been recorded in China [49]. FBNYV occurs in many Arab countries of West Asia, North and East Africa and Europe (Syria, Jordan, Ethiopia, Egypt, Algeria, Morocco, Sudan, Azerbaijan, Tunisia and Spain) [20, 21,

Table 2 Taxonomic distribution of members under the family *Nanoviridae*

Genus	Species	Acronym
<i>Babuvirus</i>	<i>Banana bunchy top virus</i>	BBTV
	<i>Abaca bunchy top virus</i>	ABTV
	<i>Cardamom bushy dwarf virus</i>	CBTV
<i>Nanovirus</i>	<i>Subterranean clover stunt virus</i> (type species)	SCSV
	<i>Faba bean necrotic yellows virus</i>	FBNYV
	<i>Faba bean necrotic stunt virus</i>	FBNSV
	<i>Pea necrotic yellow dwarf virus</i>	PNYDV
	<i>Milk vetch dwarf virus</i>	MDV
Unassigned species	<i>Coconut foliar decay virus</i>	CFDV

Recognised species is in italic

44, 48, 54, 56–59, 72]. FBNSV has been recently reported from Ethiopia [25] and Morocco [1], and PNYDV from Germany [26].

Hosts and Vectors

Most of the members under *Nanoviridae* have limited host range and are aphid-borne except, CFDV, which is

transmitted by a plant hopper, *Myndus taffini* (Table 3). They are transmitted in a circulative, nonpropagative and nontransovarial manners [22, 59]. These viruses generally multiply in phloem tissues and are not transmitted mechanically or through seeds.

Babuviruses infect limited monocot species under family Musaceae and Zingiberaceae, and are not known to infect any legume hosts. Babuviruses have limited aphid vectors, *Pentalonia nigronervosa* and *Micromyzus kalimpongensis* [6, 52, 96]. Nanoviruses naturally infect legumes (dicots), and are vectored by several aphid species. *Aphis craccivora* appears to be the major natural vector of FBNYV and MDV as it is the most abundant aphid species on legume crops and the most efficient vector under experimental conditions. In addition to *A. craccivora*, other aphid species such as *A. gossypii*, *A. fabae*, *Acyrtosiphon pisum*, and *Megoura viciae* are able to transmit MDV at various efficiencies. SCSV has been reported to be vectored by *A. gossypii*, *M. persicae* and *Macrosiphum euphorbiae* [29].

Genome Organisation

The basic genome structure of the members under *Nanoviridae* has some similarities to geminivirus. Each circular

Fig. 1 Global distribution of multicomponent ssDNA plant viruses. *b* *Banana bunchy top virus* (BBTV) (*b*1 Australia, *b*2 Fiji, *b*3 Tonga, *b*4 Samoa, *b*5 Vietnam, *b*6 Indonesia, *b*7 Taiwan, *b*8 Philippines, *b*9 Sri Lanka, *b*10 India, *b*11 Pakistan, *b*12 China; *b*13 Egypt, *b*14 Burundi, *b*15 Angola, *b*16 Gabon, *b*17 Hawaii); *a* *Abaca bunchy top virus* (Philippines), *ca* *Cardamom bushy dwarf virus* (India), *s* *Subterranean*

clover stunt virus (Australia), *m* *Milk vetch dwarf virus* (MDV) (*m*1: Japan; *m*2: China), *f* *Faba bean necrotic yellows virus* (FBNYV) (*f*1 Spain, *f*2 Morocco, *f*3 Tunisia, *f*4 Egypt, *f*6 Sudan, *f*7 Ethiopia, *f*8 Jordan, *f*9 Syria, *f*10 Azerbaijan), *cf* *Coconut foliar decay virus* (CFDV) (Vanuatu), *p* *Pea necrotic yellow dwarf virus* (Germany), *fs* *Faba bean necrotic stunt virus* (*fs*1 Morocco, *fs*2 Ethiopia)

Table 3 Insect vectors of nanoviruses and babuviruses

Name of the virus ^a	Insect vector	Mode of transmission	References
BBTV	Aphid (<i>Pentalonia nigronervosa</i>)	Persistent	[52]
ABTV	Aphid (<i>Pentalonia nigronervosa</i>)	Persistent	[70]
CBDV	Aphid (<i>Pentalonia nigronervosa</i>)	Persistent	[96]
CFDV	Plant hopper (<i>Myndus taffini</i>)	Semi-persistent	[41]
FBNYV	Aphid (<i>Aphis craccivora</i> , <i>Acyrtosiphon pisum</i> , <i>Aphis fabae</i>)	Persistent	[21]
FBNSV	Aphid (<i>Aphis craccivora</i> , <i>Acyrtosiphon pisum</i>)	–	[25]
PNYDV	Aphid (<i>Acyrtosiphon pisum</i>)	–	[26]
MVDV	Aphid (<i>Aphis craccivora</i> , <i>A. gossypii</i> , <i>A. fabae</i> , <i>Acyrtosiphon pisum</i> and <i>Megoura viciae</i>)	Persistent	[38]
SCSV	Aphid (<i>Aphis gossypii</i> , <i>Myzus persicae</i> and <i>Macrosiphum euphorbiae</i>)	Persistent	[29]

^a Full name of the virus as is in Table 2

ssDNA component contains one open reading frame (ORF) in virion sense strand and the noncoding intergenic region with a stem-loop structure (Fig. 2). The loop containing a conserved nona-nucleotide sequence is generally identical among the DNA components of a virus and has minor variation among the members. The stem and loop structure is the origin of replication [93]. The DNA components encoding the major viral proteins are replication associated protein (Rep = DNA-R), coat protein (CP = DNA-S), cell-cycle link protein (Clink = DNA-C), movement protein (MP = DNA-M) and a nuclear shuttle protein (NSP = DNA-N) (Table 4). Each coding region is preceded by a promoter sequence with a TATA box and followed by a poly-A signal. The replication of *Babuvirus* and *Nanovirus* is through rolling circle mechanism. In many of the *Nanovirus* or *Babuvirus*, several ssDNA components have been shown to encode Rep related proteins, of which only one Rep component that controls replication of other ssDNA components is known as master Rep (M-Rep) [94]. Replication of the DNAs is facilitated and enhanced by the action of 19 kDa Clink, a cell cycle linked protein [4].

Up to 12 distinct DNA components have been isolated from plants infected by different nano- or babuviruses. Only a single CP has been found to be associated with the viral capsid, which is about 19 kDa [100]. All the *Nanovirus* DNAs code for only a single protein except, in BBTV, where the M-Rep encoding ORF contains additional 5 kDa protein of unknown function. Being multicomponent virus, the number of ssDNA components that are required for inducing disease was not known until 2006, when Timchenko et al. [95] demonstrated infectivity of the cloned DNAs of FBNYV through agroinoculation. Only five DNA components DNA-R, DNA-S, DNA-M, DNA-U1 and DNA-U2 were enough to produce disease, however, the progeny viruses could not be transmitted through aphid vectors. The first comprehensive

demonstration of infectivity of the cloned DNAs was shown for FBNSV. A group of eight cloned DNA components produced typical disease symptoms as well as biologically active progeny virus that was successfully transmitted through aphid vector [25].

Disease and Virus Description

Bunchy Top of Banana

Bunchy top of banana is the one of the earliest known and significant plant viral diseases. Banana being vegetatively propagated, the disease spreads widely through planting material. The banana bunchy top disease was first observed of in Fiji in 1889. It emerged as a serious threat to banana cultivation in many countries, including Fiji in 1927, Egypt and India in 1953, the Philippines and Taiwan in 1961, Tonga and Samoa in 1967, China in 1979, Pakistan and Hawaii in 1989 [2, 16]. In Australia, the banana industry in south-east Queensland and northern New Wales was virtually destroyed by the bunchy top disease in 1920 [16]. In India, the disease was introduced from Sri Lanka during 1940 through southern India and now it has spread all over the banana growing region. Of all the known banana viruses, BBTV is the most serious viral pathogen of banana.

Initial disease symptoms are not very conspicuous. The characteristic symptoms are produced at the advanced stage of infection. Initially, dark green streaks appear on petiole, midrib and veins of the leaves. Finally, the affected plants become stunted containing a bunch of short, narrow, erect and chlorotic leaves, which gives bunchy appearance on the crown of the plant (Fig. 3c). The severely affected plant does not produce fruits and dies eventually resulting in a total loss. The virus is naturally transmitted by aphid, *Pentalonia nigronervosa* in a persistent manner and

Fig. 2 Genome organization of multicomponent ssDNA plant viruses. *M-Rep* master Rep, *CP* coat protein *MP* movement protein, *NSP* nuclear shuttle protein, *C* cell-cycle linking protein, *U1–U3* unknown function; * stem and loop structure, *solid triangle* TATA box, *dot* poly-A signal. In *Babuvirus*, *hatched box* indicate major common region. No ORF was identified in U3 component of Abaca bunchy top virus. **a** Nanovirus, **b** Babuvirus

Table 4 Genome components of the viruses under *Nanoviridae*

Designation of DNA component	DNA component number ^a					Protein function
	FBNYV	MDV	SCSV	BBTV	ABTV	
DNA-R	2	11	8	1	+	Replication
DNA-S	5	9	5	3	+	Encapsidation
DNA-C	10	4	3	5	+	Cell-cycle link protein
DNA-M	4	8	1	4	+	Movement protein
DNA-N	8	6	4	6	+	Nuclear shuttle protein
DNA-U1	3	5	7	–	–	Unknown
DNA-U2	6	7	–	–	–	Unknown
DNA-U3	–	–	–	2	+	Unknown
DNA-U4	12	–	–	–	–	Unknown

^a The *number* indicates the designation of specific DNA components isolated

+Two clones were isolated (one each from two isolates of ABTV) for each of the genome component. Specific number was not used to designate each DNA component

through vegetative propagation of sucker from infected plants [51, 52]. Vector transmission rate is directly proportional to the number of viruliferous aphids and inversely

proportional to the age of plant [102]. Transmission of BBTV by *P. nigronevosa* is influenced by temperature, vector life stage and plant access period [3].

Fig. 3 Disease symptoms of multicomponent ssDNA plant viruses. **a** *Milk vetch dwarf virus* (MDV) in faba bean showing yellowing; **b** Cardamom bushy dwarf virus in large cardamom showing excessive stunting and proliferation of lateral shoots; **c** *Banana bunchy top virus* (BBTV) in banana showing bunchy crown; **d** Coconut foliar decay virus (CFDV) in coconut (Malayan Red Dwarf) showing yellowing of fronds; **e** *Faba bean necrotic yellows virus* (FBNYV) in faba bean showing yellowing and stunting; **f** FBNYV in faba bean leaves showing marginal chlorosis and necrosis. (Source of figures: BBTV on banana was contributed by Dr. VK Baranwal, Indian Agricultural Research Institute, India; MDV on faba bean by Dr. Y Sano, Niigata University, Japan; FBNYV on faba bean by Dr. SG Kumari, ICARDA, Syria; CFDV on coconut by Dr. JW Randles, University of Adelaide, Australia)

BBTV

Determination of the causal virus of banana bunchy top ran in to confusion for a while. Research progress on BBTV was slow compared to the other important plant viruses. Purification of the virus from infected banana plants is difficult due to low concentration of virus and difficult tissue type. Initially, BBTV was considered as a possible member of the luteovirus on the basis of yellowing symptoms, cytopathological effect on phloem and aphid transmission [17, 63]. During 1989–1990, the reports of virus purification from bunchy top affected banana were published by two independent groups of worker from Gabon (Africa) and Taiwan [39, 91]. The African group reported presence of isometric virus of 28 nm and the Taiwan group reported 20–22 nm isometric virus. Further, observation of dsRNA was observed with the diseased plant [17] and ssRNA with the purified preparation [103] supported the idea of luteovirus association.

The first successful demonstration of association of ssDNA virus with the disease was from two laboratories in Australia in 1991 [31, 92]. They concluded that the BBTV virions were isometric with a diameter of 18–20 nm with buoyant density of 1.28–1.30 g/ml in caesium sulphate and

sedimentation coefficient of 46S in isokinetic sucrose density gradients. The A_{260}/A_{280} of purified preparations was about 1.33. The purified virus preparation contained ssDNA of about 1 kb and a single coat protein M_r 20500–20100. Both the polyclonal antibodies and DNA probe prepared from the purified virus detected BBTV by ELISA in field-infected banana plants and in aphid [31, 92].

The first genome sequence information was generated in 1993 on component-1 DNA of BBTV genome, which was circular ssDNA consisting of 1111 nucleotides encoding one large ORF of 858 nucleotides in virus strand encoding a protein of 286 amino acids with a M_r of 33.6K. The protein was identified as a replicase based on the presence of a dNTP binding motif (GGEGKT) [32]. The noncoding region contains a stem and loop structure. The loop contains nonanucleotide sequence (TATTATTAC), which was nearly identical to that of geminivirus (TAATATTAC) and CFDV (TAGTATTAC). The sequence of BBTV component-1 was not significantly similar to any plant DNA viruses except CFDV [82] and SCSV [14]. The component-1 DNA was detected in all bunchy top affected plant samples from 10 different countries indicating an integral part of BBTV genome. Based on component-1 sequence,

existence of two groups of BBTV isolates were evident: the South Pacific group containing isolates from Australia, Burundi, Egypt, Fiji, India, Tonga and Western Samoa; and the Asian group containing isolates from the Philippines, Taiwan and Vietnam [43]. The mean sequence difference within each group was 1.9–3.0% and between the isolates from the two groups was about 10%. BBTV occurring in Indian [99] and Pakistan [2] has been characterized based on sequence of 5–6 components of DNA and both the isolates belonged to the South Pacific group. Phylogenetic analysis of major common region of all the six DNA components of BBTV showed a possible concerted evolution within the Pacific group due to recombination in this region [37].

During 1994–1995, additional DNA components were cloned and sequenced, which provided the evidence that BBTV was a multi-genomic virus containing at least seven ssDNA components [9, 10, 105, 106]. The DNA components of BBTV have potential source of promoters. Assessment of DNA components 1–6 showed that the promoters are functional in both monocot and dicot plants [19]. Promoter from DNA component-2 showed greater efficacy compared to 35S promoter from *Cauliflower mosaic virus*.

The functions of various DNA components of BBTV have been addressed. DNA component-1 encoded a Rep protein [10, 32, 37]. Two mRNA transcripts were identified from component-1 of BBTV; larger one transcribed from Rep ORF and the shorter transcript mapped completely internal to Rep ORF [7]. The smaller transcript encoding 5 kDa protein of unknown function is unique and not known in the corresponding DNA component of nanoviruses. The DNA component-3 was shown to encode the CP [100]. The protein encoded by component-4 was found to localised in cell periphery, whereas, the protein encoded by component-3 and -6 were in both nucleus and cytoplasm. The component-6 was identified to encode a NSP, while the component-4 to encode a movement protein [101]. The characteristics of the proteins encoded by BBTV DNA-4 and DNA-6 are very similar to those of the BC1 (movement) and BV1 (nuclear shuttle) proteins, respectively of *Squash leaf curl virus* and other begomoviruses [67, 85]. Based on yeast two-hybrid analysis and occurrence of LXCXE motif, component-5 was identified to encode the clink, a retinoblastoma-binding like protein [101].

Three possible satellite DNAs have been isolated from BBTV infected banana; BBTV S1 [35], BBTV Y/X [104, 106] and BBTV S2/W2 [35, 104]. Unlike, BBTV DNA component-1 to -6, S1 and S2 appear to be prevalent in Asian group of isolates rather than in South Pacific isolates [35]. Unlike component-1, these satellite DNA components are not mandatory for the replication of the BBTV. The DNA component-1 is the minimal replicative unit of

BBTV and is required for replication of other components and therefore, it was identified as the M-Rep [36]. Three iteron with sequence GGGAC (F₁, F₂ and R) have been identified in the intergenic region of DNA components of BBTV. Interaction of Rep with the iteron is essential for efficient DNA replication [34].

Bunchy Top of Abaca

Abaca or Manila hemp (*Musa textilis*) is a fiber crop mainly grown in Philippines. The fiber is mainly used in fabrics, bank notes, lens-cleaning tissue, tea bags and capacitor papers [80]. About 75% of the total abaca fiber is produced in the Philippines. In the Philippines, abaca bunchy top disease was first broke out in 1915 in Cavite and Silang provinces and by 1980, the disease spread in Bicol, a major abaca region [68, 69]. The disease occurs in all major production areas and is a major factor in the decline of abaca export from the Philippines [79, 80]. The quantitative estimate of losses due to bunchy top is not available; however, the disease is very destructive. In Cavite province, about 12000 ha of abaca was wiped out in 1928 [11].

The symptoms of the abaca bunchy top disease include presence of chlorotic areas on the youngest furled leaf, progressive narrowing and shortening of the new leaves, dark green flecks or vein clearing of the minor leaf veins and chlorosis of leaf margins. The false stem becomes stunted but the girth of the upper end may be same or greater than the lower end. The infected abaca plants survive for about two years, gradually become smaller and finally all of the leaves and leaf sheaths turn brown and die. The disease is naturally transmitted by the banana aphid, *Pentalonia nigronervosa* [68].

ABTV

Bunchy top disease of banana and abaca were considered to be caused by a same virus due to similar symptoms and transmission [53, 70]. The disease in abaca could be induced by BBTV isolated from banana (Magee, 1927). But Sharman et al. [87] reported that the virus causing abaca bunchy top disease is distinct from BBTV. The viruses were serologically distinct and shared only 79–81% amino acid sequence identity of the putative coat protein. Overall nucleotide sequence identity with BBTV across all components ranges between 54–76%. In the phylogenetic analyses, ABTV and BBTV each located together but on separate branches separating from nanoviruses. The genome of ABTV consisted of at least six circular ssDNA of about 1.0–1.1 kb each. However, unlike BBTV, ABTV isolate lack an internal ORF in the DNA-R component.

Foorkey of Large Cardamom

Foorkey disease of large cardamom (*Amomum subulatum*) is known since 1936 in Darjeeling hills, India [66]. The affected plants result into profuse vegetative growth of excessively stunted tillers from the rhizome (Fig. 3b). The stalks are greatly reduced and the leaves become pale green and slightly curled. The diseased plants survive for few years and remain unproductive. The foorkey disease was considered to be caused by a virus different from those causing bunchy top of banana and katte disease of small cardamom (*Elettaria cardamomum*) [96]. The foorkey is not sap or seed transmissible, however it is transmitted by the banana aphid, *P. nigronevosa* in a persistent manner [96]. Basu and Ganguly [6] reported that at Kalimpong, the foorkey was transmitted by another aphid, *Mycromyzus kalimpongensis*, but not by the banana aphid. The virus could be transmitted to both large and small cardamom, but not to *Musa sapientum*, *Gladiolus* sp, *Canna indica*, *Zingiber officinale*, *Triticum aestivum*, *Sorghum vulgare* and *Zea mays* [96].

CBDV

In leaf-dip electron microscopy, hardly any virus like structure is visible in the foorkey affected large cardamom samples. Partially purified preparation contained a few isometric particles of 17–20 nm. ELISA with polyclonal antisera to BBTV showed weak serological reaction. Nucleotide sequence of Rep gene showed 80–82% identity with that of BBTV and 47.6–48.5% identity with the nanoviruses. Based on this information along with the available biological properties, association of a new *Nanovirus*, Cardamom bushy dwarf virus (CBDV) was identified with the foorkey disease of large cardamom [60]. Six distinct full-length DNA components encoding putative master-Rep (M-Rep), non-master-Rep, coat protein and three DNA components of unknown function (FrU1, FrU2 and FrU3) were cloned and sequenced, which showed phylogenetic affinity with babuviruses [61, 84].

Stunt of Subterranean Clover

Subterranean clover (*Trifolium subterraneum*) and several other pasture and grain legumes (pea, French bean and broad bean) in Australia are affected by a stunt disease [28, 29, 90]. The disease was initially observed in New South Wales, Tasmania, Victoria and Queensland. The disease caused yield losses of up to 65% [28]. The characteristic symptoms of the disease are severe stunting with marginal yellowing and cupping of young leaves and reddening of older leaves.

SCSV

Initially, the disease was thought to be caused by mineral deficiency [65]. Subsequently, it was shown to be an aphid transmitted virus [28, 29]. SCSV was persistently transmitted by *Aphis craccivora* and *Myzus persicae*. However, it was not sap or seed transmissible. Novel virus-like isometric particles of 17–19 nm were purified from subterranean clover and pea plant affected with the stunt disease [13]. Purified SCSV particles did not induce disease on subterranean clover inoculated with aphid (*Aphis craccivora*) fed on the purified preparation [14]. The virus particles had a $T = 1$ capsid structure containing 60 polypeptide subunits each with M_r of 19,000. SCSV has a functionally divided genome composed of multiple circular, ssDNA molecules of about 1 kb, each of which are encapsidated in a separate particle with a short complementary primer sequence [13, 14]. The sequence of eight circular, ssDNA components of SCSV has been determined so far [8, 94]. All components contain a single ORF in sense direction and a stable stem-loop structure in the noncoding region. The loop contains a conserved nonanucleotide sequence (TAGTATTAC).

Necrotic Yellows of Faba Bean and Other Legumes

Necrotic yellows are important viral diseases of several important food and fodder legumes in Western Asia and North Africa [55]. Faba bean (*Vicia faba*) is an important crop affected by the disease. In addition, several cool season (*Lens culinaris*, *Cicer arietinum* and *Pisum sativum*) and summer season legumes (*Phaseolus vulgaris* and *Vigna unguiculata*) are also affected by the disease [20, 44, 54, 58]. The disease (Fig. 3e, f) causes yellowing, rolling and necrosis of leaves and stunting and death of plants. The disease was first observed in Syria in 1988. During 1991–1992 and in 1998, the disease caused complete crop failure in faba bean in Egypt [55, 58]. The disease was persistently transmitted by various aphid species, of which *Aphis craccivora* was the most significant natural vector [22]. The disease could be transmitted through *A. craccivora* to a new host, *Arabidopsis thaliana* [97].

FBNYV

Isometric virus of 18 nm containing circular ssDNA as genome of 1.0 kb was observed in faba bean affected with necrotic yellows and the virus was named as FBNYV [44]. By host range, transmission and serology, the virus was similar to SCSV and MDV. In 1995, the first sequence information was obtained for the component-1, which contained 1002 nucleotides encoding 32.3 kDa Rep protein [45]. The sequence information showed that the FBNYV is

a distinct virus with similarities to other nanoviruses. Five more components (C2–C6) of FBNYV were determined; C2 encoded an additional Rep of 33.1 kDa, C4 encoded MP, C4 and C5 encoded 19 kDa CP and C3 and C6 encoded proteins of unknown function [46]. Further, additional four components (C7–C10) of ssDNA were isolated and characterized [47]. The C7 and C9 encoding Reps were similar to C1 and C2. C8 encoded 17.4 kDa protein of unknown function and C10 encoded Clink protein. A total of 12 components were characterised for FBNYV and this provided evidence that FBNYV was a multicomponent ssDNA virus and a distinct species under the genus *Nanovirus*. Five different DNA components (C1, C2, C7, C9 and C11) are known to encode different but closely related Rep in FBNYV. Only, C2 was shown to support replication of all the six other genome components. Further, only the C2 DNA was always detected in 55 samples of FBNYV from eight countries. These provided evidence of existence of a master replication protein (M-Rep) encoded by FBNYV [93].

In 2000, Aronson et al. identified the function of C10 component, which encoded a 20 kDa protein that interacted with cell-cycle regulators retinoblastoma-like proteins (pRB). The FBNYV-C10 encoding protein has been named as Clink protein due to its potential to link viral DNA replication with key regulatory pathways of the cell-cycle. Clink significantly increases replication of FBNYV DNA. The interaction of Clink with pRB and SKP1 has been demonstrated in yeast two-hybrid system and in vitro by pull-down assays with the proteins expressed in *E. coli* [4]. Similar interaction occurring in plant was demonstrated by FBNYV-based expression vector system [5].

FBNYV or any other nanoviruses contains multicomponents of ssDNA. It was not known that how many components were necessary for causing disease. Timchenko et al. [95] for the first time demonstrated infectivity of the FBNYV cloned DNAs. Only five DNAs, DNA-R, DNA-S, DNA-M, DNA-U1 and DNA-U2 were sufficient to induce disease symptoms in *Vicia faba* following agroinoculation. The inoculated plants with FBNYV DNAs produced virions but they were not transmitted by *Aphis craccivora* or *Acyrtosiphon pisum*—the two efficient aphid vector of FBNYV.

Necrotic Stunt/Dwarf of Faba Bean and Pea

Faba bean necrotic stunt was first observed in Ethiopia [23] and subsequently recorded in Morocco [1]. The virus causing the necrotic stunt in faba bean has been identified as a new *Nanovirus* species, FBNYV. Eight distinct DNA components were characterised and inoculation with these cloned DNAs produced typical disease in faba bean. The progeny virus of the cloned DNAs was transmissible by its

vector, *Aphis craccivora* and *Acyrtosiphon pisum* [25]. This is the first conclusive demonstration about the infective unit of *Nanovirus* genome.

Pea necrotic yellow dwarf was first observed in 2009 in Germany [26]. The disease is characterized by severe yellowing, necrosis and stunting of plant and is transmitted by pea aphid, *Acyrtosiphon pisum* but not by sap. A new *Nanovirus* species, PNYDV was identified to be associated with the disease. PNYDV is serologically different from other nanoviruses. Only Rep encoding DNA component has been sequenced, which shared 73–79% sequence identity with that of FBNYV, FBNSV, MDV and SCSV [26].

Dwarf of Milk Vetch and Other Legumes

Yellowing and dwarfing of Chinese milk vetch (*Astragalus sinicus*), a common green manure crop was first reported in Japan [38, 62]. The disease is transmitted by *Aphis craccivora* and known to affect other legumes, faba bean (Fig. 3a), broad bean, pea and soybean. Now, MDV is known to occur in China [49].

MDV

Initially, MDV was thought to be a luteovirus based on symptoms, vector and presence of 25 nm virus particles [71]. The correct identity as a *Nanovirus* was evident in 1998, when the genome sequence information was obtained [86]. Prior to this, *Nanovirus* like virus particles of 18 nm containing circular ssDNA of 1 kb were obtained in pea leaves [40]. Ten ssDNA components of MDV have been cloned and sequenced; these are designated as C1–C10 [86]. The DNA components of MDV were similar to SCSV and FBNYV.

Analysis of the predicted amino acid sequences revealed that C1, C2, C3 and C10 all encoded distinct Rep proteins of 33 kDa, which show only 42–57% amino acid identity. MDV-C7 and FBNYV-C6 each encode a 15 kDa protein, which was not present in SCSV and BBTv. C11 (DNA-R) encoded the M-Rep, required for replication of all the other genomic DNAs [94]. Based on the sequence similarity between proteins of different nanoviruses, C4, C6, C8 and C9 were identified to encode Clink, NSP, MP and CP, respectively [98]. The function of the proteins encoded by C5 (DNA-U1) and C7 (DNA-U2) is unknown. The promoter activity of the MDV DNA components (C1–C11) has been characterized [88]. The components, C4–C8 showed stronger GUS activity compared to 35S promoter. The M-Rep and other Rep encoding components (C1, C2, C3, C10 and C11) showed low level of promoter activity.

Foliar Decay of Coconut

The foliar decay, a lethal disease was first observed in introduced varieties of coconut (*Cocos nucifera*) palms in Vanuatu, formerly New Hebrides during 1979–1980 [12, 18, 41]. Symptoms (Fig. 3d) are characterized by appearance of yellowing in several leaflets of the fronds between 7 and 11 positions down the crown from the unopened spear leaf. This is followed by yellowing, necrosis and death of fronds and crown. The dead fronds hang downward from the petiole and other fronds become yellow and plants die eventually.

The affected plant produces inflorescence that develops normal but a fewer nuts. Susceptible trees usually die within 1–2 years of the appearance of symptoms. The local variety, ‘Vanuatu Tall’ is symptomless and the hybrids developed from this variety exhibit mild symptoms [12, 42]. The cultivar, Malayan Red Dwarf is highly susceptible and is used as an indicator plant for epidemiological and insect transmission studies. The virus was not transmitted by seeds or pollen, but by the plant hopper, *Myndus taffini*. The plants inoculated through vector, *M. taffini* produce symptoms at 6–11 months post inoculation [41, 42].

CFDV

The etiology of coconut foliar decay disease was a puzzle for a long time. No fungi, bacteria and nematode were associated with the disease [42]. No virus or microorganism was detected by electron microscopy and detection of coconut cadang cadang viroid was also negative [74]. Association of a single-stranded DNA with sedimentation coefficient <75S and buoyant density of about 1.36 g/ml in Cs₂SO₄ was reported with the coconut plant showing foliar decay disease induced by feeding of *M. taffini* [75, 76]. Association of isometric virus particles of 20 nm with the foliar decay disease was first observed by Randles and Hanold [73]. Later on, Rohde et al. [82] have characterized a ssDNA component associated with the CFDV by cloning and sequencing. The ssDNA molecule contained 1291 nucleotides with the potential of at least six ORFs, one of which (ORF1) coded for a leucine-rich protein (33.4 kDa) with the nucleoside triphosphate-binding motif GXGKS indicating a Rep protein. The DNA sequence contained a stable stem and loop structure, which resembles to that of geminiviruses. The DNA sequence data, however, did not resemble any known taxonomic group of plant viruses. CFDV is localized within the phloem of coconut palms. Multiple sampling is needed for reliable diagnosis as CFDV as it is distributed unevenly in whole plants in a non-symptomatic manner, and a minimum sampling should include tissue from fronds 1, 3 and 5, and secondary roots [77].

To determine the promoter activity of CFDV DNA in plant, reporter gene was fused to the CFDV DNA and tobacco plants were transformed. The expression of the reporter gene was obtained in the phloem tissue of the vascular system in stem, leaves and flower demonstrating the association of CFDV DNA with the phloem [83]. The *cis*-signals involved in CFDV promoter strength and tissue specificity were identified and it was shown that, CFDV stem-loop structure dramatically influences transcriptional efficiency [33]. The Rep protein of CFDV was expressed as a 6× His recombinant protein and it was demonstrated that the protein possessed sequence non-specific RNA- and ssDNA-binding activities as well as magnesium-dependent ATPase/GTPase activity [64]. The Rep protein was also able to interact with itself and associated predominantly with nuclei and membranes of infected/transfected cells [64]. The Rep activity of CFDV was very similar to those reported for Reps of geminiviruses.

Concluding Remarks

The family *Nanoviridae* containing multigenomic small isometric plant viruses is relatively young and is expanding. The family includes two distinct groups of members that are divided into two genera, *Nanovirus* and *Babuvirus* [98]. The name *Nanovirus* was originally coined to represent small isometric multigenomic plant viruses. Now, *Nanovirus* refers to only one of the genera and therefore no more represents as a common name for the members under the family. As the members under the present genus, *Nanovirus* have a common character of infecting legumes, a new name of this genus, ‘Lenovirus’ derived from legume *Nanovirus* may be more meaningful instead of *Nanovirus*.

Multicomponent ssDNA plant viruses although were identified in 1990s, many of them are known to exist for a long time as they are associated with historic plant diseases and some of them have recently emerged [25, 26]. Genomics of these viruses opened up new area of studies that they are the source of promoters [19, 83, 88] for gene expression in plant and vectors that provide efficient expression system for studying *in planta* protein-protein interactions [5].

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