

Mini-Review

## The Genera *Babuvirus* and *Badnavirus* in Asia

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In the plant virus world, there are six genera of plant viruses with dsDNA genomes and six genera with ssDNA (Fauquet et al., 2005). The dsDNA viruses are comprised of 4 genera in the *Caulimoviridae*, the genus *Badnavirus* and the genus *Tungrovirus*. The ssDNA viruses are comprised of four genera in *Geminiviridae*, and the two genera *Nanovirus* and *Babuvirus* in the *Nanoviridae*. The genera *Babuvirus* and *Badnavirus* are not well studied in Asia. However, we recognized the significance of two species, *Banana bunchy top virus* (BBTV) in the genus *Babuvirus* and *Banana streak virus* (BSV) in the genus *Badnavirus*, during the survey of banana viruses in Asia. Their main characters will be introduced in this mini-review.

**Keywords :** *Babuvirus*, *Badnavirus*, banana, *Banana bunchy top virus*, *Banana streak Mysore virus*

**Bananas and their virus diseases.** Bananas and plantains (*Musa* spp., referred to as bananas hereafter) are the main and almost exclusive hosts of *Banana bunchy top virus* (BBTV) and *Banana streak virus* (BSV). They are grown mainly in humid tropical and subtropical countries, and are important as staple crops as well as a commodity export crop. Bananas originate from the Indo-Malaysian region. Two main genomic groups designated as *M. acuminata* (AA) and *M. balbisiana* (BB) and their progeny, with various type of crossing such as haploid and triploid, are grown. In the Philippines, *M. textilis* (abaca) is cultivated as an industrial crop to produce textiles. In Japan, over 1 million tons of bananas were imported per year in 2005 and 2007. Banana production in Japan is small scale and limited mainly to the Okinawa prefecture. In the Okinawa prefecture, *M. balbisiana* var. *liukuensis* is also grown for production of traditional and high-value textiles. Because of the close historical and geographical relationships between Asian countries, the origin of banana viruses in this prefecture was thought to be an important target of study. In our investigation on banana viruses in Thailand, the

Philippines, Indonesia, Vietnam, Japan and Myanmar, we detected 4 major viruses: *Cucumber mosaic virus* (CMV), *Banana bract mosaic virus* (BBBrMV), BBTB and BSV (Jones, 2000; Wardlaw, 1972). The most common and serious virus was BBTB (Furuya et al., 2004, 2005, 2006).

**Genus *Babuvirus*.** BBTB is the type and single species of genus *Babuvirus*. The genus *Nanovirus* which is closely related to the genus *Babuvirus*, accommodates *Subterranean clover stunt virus* (SCSV) as type species and other 2 species; *Faba bean necrotic yellows virus* (FBNYV) and *Milk vetch dwarf virus* (MDV).

The species of *Nanoviridae* are characterized by non-enveloped spherical virions of 17-20 nm in diameter. Virions are limited to the phloem tissue of infected plants. They are transmitted by aphids in a persistent but not propagative manner. As opposed to BBTB, geographical distribution of other species is limited: FBNYV is limited to West Asia to Africa and Spain; SCSV is found only in Australia; MDV only in Japan (Fauquet et al., 2005). Molecular characterization of MDV as an Asian nanovirus has been conducted by Sano et al. (1998).

**Discovery and occurrence of *Banana bunchy top virus*.** Bananas infected by BBTB show dash-like streaks as the first observable symptoms. As the disease progresses the leaf blades become narrow, and plants show symptoms such as stunting and bunched leaves. The fruits, if any, are malformed. Finally, the disease (banana bunchy top disease; BBTD) results in plant death (Fig. 1A). Because of this direct influence on productivity, BBTB is considered to be the most economically destructive disease of banana. The disease is widespread in Asia including China and Japan, Africa and Oceania but not in Central and South America (Dale, 1987). It is transmitted in a persistent manner only by banana aphid (*Pentalonia nigronervosa*) (Fig. 1B), and through vegetative propagation, but not by artificial manual inoculation. In the Philippines, the pathogen of bunchy top disease of abaca had been designated as abaca bunchy top virus (ABTV). According to the molecular analysis, BBTB is detected from bunchy top affected abaca and thus ABTV can be confirmed as the synonym of BBTB (Furuya et al.,

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Fig. 1. Symptom of banana bunchy top disease (BBTD) (A) and *Pentalonia nigronervosa* (B).

2006).

**Genome organization of BBTV.** BBTV has a multiple component genome consisting of 6 circular ssDNA components (1-1.1 kb), DNA-R(1), -S(3), -M(4), -C(5), -N(6) and -U3(2). The numbers attributed to each ssDNA component are a previously used designation (Fig. 2) (Harding et al., 1993). Each of the components contains a common stem-loop region (69 nts) and major common region (CR-M, 66-92 nts) as conservative regions, a TATA box and a poly(A) signal. The coat protein (CP) consists of 170 aa (19.6 kDa) and is encoded by DNA-S. The movement protein (MP) is encoded by DNA-M (Burns et al., 1994; Wanitchakorn et al., 1997, 2000). Satellite or satellite-like DNAs with sequence(s) resembling BBTV DNA-R have been reported in Taiwan and subsequently in other countries (Yeh et al., 1994).

The major differences between babuviruses and nanoviruses are the number of DNA components and the host range. Moreover, they share no serological relationship and have different aphid vectors. An important similarity is that both BBTV and nanoviruses have gene(s) that correspond

with the genus *Geminivirus* (Burns et al., 1995; Hafner et al., 1997).

**Diversity of BBTV and its onset in Japan.** Karan et al. (1994) used comparison of nucleotide sequences of DNA components (Table 1) to classify BBTV into two major groups: Asian and South Pacific.

The intragroup difference was approximately 3% and the intergroup difference approximately 30%. The higher variability among the sequences of the Asian group BBTVs than those of the South Pacific group BBTVs suggest that the outbreak of BBTV occurred much earlier in Asia. The Asian group includes isolates from China, Vietnam, Indonesia, the Philippines, and Taiwan. The South Pacific group includes isolates from Australia, the Pacific Islands, India, Pakistan, Egypt and some countries in Africa. More recently some BBTV isolates from Myanmar were found to be grouped into the South Pacific group (Nagashima, personal communication). Su et al. (2003) demonstrated the differentiation of BBTV strains with distinct geographic origins and symptoms using molecular and biological techniques.

As for BBTV in Japan, after the first report of BBTD in

Table 1. Differentiation of two BBTV groups

Group	Locality
South Pacific	Fiji, Australia, Burundi, Tonga, Egypt, India, Hawaii*, Pakistan**, Myanmar***
Asian	Philippines, Indonesia****, Japan*****, China*****, Taiwan Vietnam

Karan et al., 1994; \*Xie et al., 1995; \*\*Shah et al., 2005; \*\*\*Nagashima et al., personal communication; \*\*\*\*Furuya et al., 2004, \*\*\*\*\*Furuya et al., 2005, \*\*\*\*\*La et al., 2001)

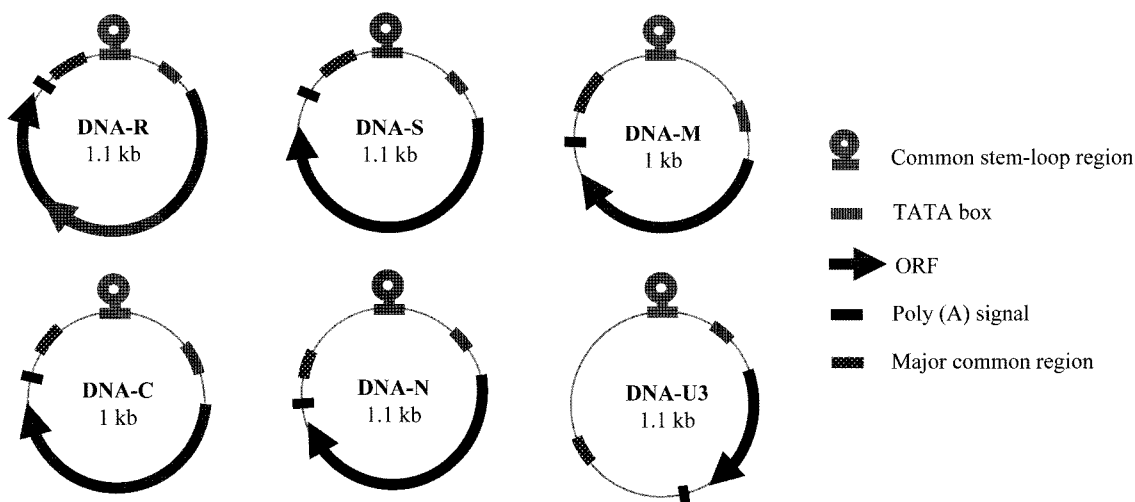


Fig. 2. Genome organization of BBTV.

1968, the confirmation of BBTV was conducted by I-SEM and ELISA (Kawano and Su, 1993). Molecular analysis of BBTVs in Japan was first carried out by Furuya et al. (2005). All isolates of BBTV from Okinawa belonged to the Asian group and formed one dense cluster with Taiwan, Indonesian and Filipino isolates). There was a less than 1% divergence of the complete DNA-R sequence among seven BBTV isolates. These isolates were collected in seven different islands stretched over 400 km in Okinawa. Compared with the previously reported high (8%) divergence in Vietnamese BBTVs (Bell et al., 2002), the small divergence among Japanese isolates suggests a recent and non-frequent invasion history of BBTV to Okinawa, Japan.

**Diagnosis and detection of BBTV.** Identification of BBTV can be via its typical symptoms. For more accurate and large scale screening, ELISA is suitable using commercially available antiserum specific to BBTV. For sensitive detection, differentiation of two groups, and confirmation of virus free in production of tissue cultured seedlings, PCR can be used. For the detection of BBTV by PCR, a set of primers (F3, FPCR4) by Karan et al. (1994) is useful.

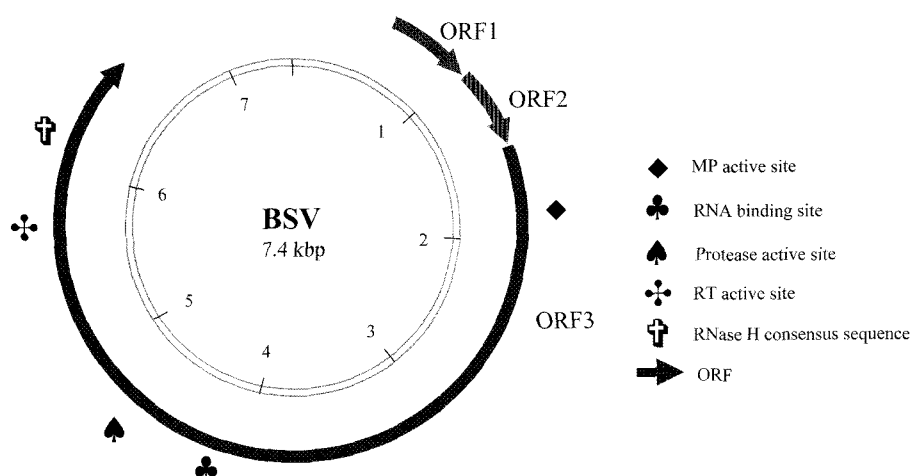
**Protection and mitigation of BBTV.** While no successful control measures have been reported, phyto-sanitation measures are recommended. These include roguing of diseased plants and re-planting of virus free banana seedlings, as well as vector control. There is no known BBTV-resistant variety. Recently, *M. balbisiana* var. *liukuensis* was found to be resistant both by field observations and artificial inoculation using banana aphids (Furuya et al., data not shown). Several evaluations of BBTV resistant lines developed by gamma radiation mutation have been started to establish resistant varieties (Dizon et al., [http://www.fnca.jp/english/mb/e\\_banana\\_meeting\\_iii.html](http://www.fnca.jp/english/mb/e_banana_meeting_iii.html)). As

the cost of BBTV mitigation will be very large, strong plant quarantine restrictions should be applied in importing plant materials e.g. suckers.

**Impact of babuvirus studies.** Though BBTV does not appear to be important in non-banana cultivating countries, studies on this virus deepen our understanding of the plant virus world. For example, the new concept of the “birth and death evolution model,” which is known in the case of eukaryotic multi-gene families, can be applied to BBTV. Based on the phylogeny analyses of nanoviruses and BBTV, Hugh (2003) suggested that the Rep<sup>-</sup> (replication initiation protein) encoding genome components of these viruses have been repeatedly duplicated and also lost during evolution. Such duplication and loss are suggested to take place independently in various BBTV populations. In the model, new genes are created by gene-duplication, and inactivation or deletion of some of these duplicated genes. Likewise, there are many more unanswered questions concerning the genus *Babuvirus*: Is BBTV the sole member of the genus *Babuvirus*? Is banana the only host of BBTV? Thus we need far more study in this genus.

**Genus *Badnavirus*.** Another important virus in banana production is BSV, in the genus *Badnavirus*, *Caulimoviridae*. Each of the bacilliform virus particles contains a single molecule of circular dsDNA with 3 ORF (open reading frame)s.

The genus *Badnavirus* consists of *Commelina yellow mottle virus* as the type species, another 17 species as member species, and at least five species as tentative species (Fauquet et al., 2005). *Canna yellow mottle virus* was the first reported *Badnavirus* in Japan in 1979 (Yamashita et al., 1985). One tentative member of the genus *Badnavirus*, Pineapple bacilliform virus, is widespread in



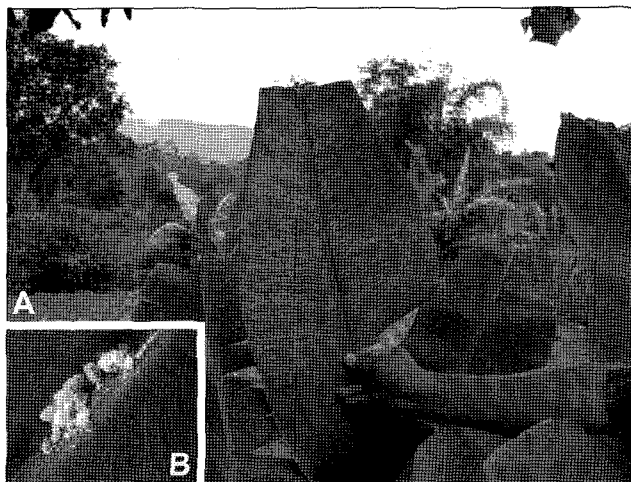
**Fig. 3.** Genome organization of BSV.

pineapple production areas (Thomson et al., 1996). The authors also detected the virus sequence from pineapples in some Asian countries including Japan (data not shown). It should be noted that many hosts of badnaviruses such as banana, yam, taro, cacao, and sugarcane grow in the tropics or subtropics and are propagated vegetatively. For some of the badnaviruses, the manner of transmission has not been well documented

The genome of the genus *Badnavirus* is one molecule of circular dsDNA (approximately 7.4 kb for BSV) (Fig. 3). The CP, aspartic protease, reverse transcriptase (RT), ribonuclease H (RNase H) and movement protein (MP) are first expressed as a large polyprotein. The polyprotein is subsequently cleaved post-translationally into functional units by the aspartic protease (Fauquet et al., 2005).

Among the species in the *Caulimoviridae*, the genus *Tungrovirus* with *Rice tungro bacilliform virus*, a serious rice virus in Asia, is most closely related to badnaviruses. They share the same virion bacilliform morphology and 20-25% identity of genome sequence.

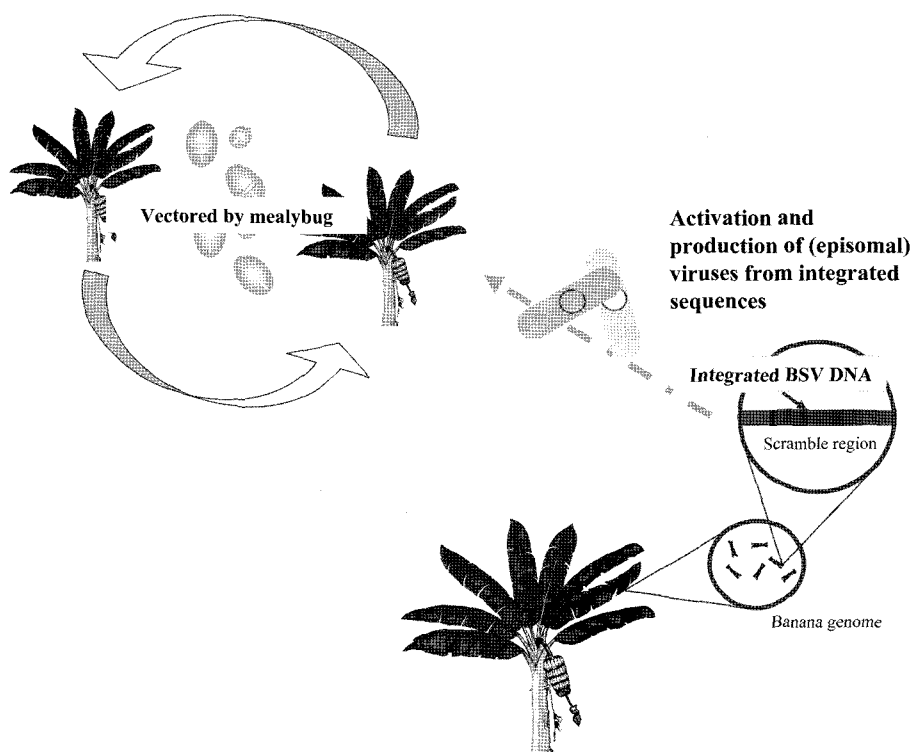
**Discovery and classification of BSV.** BSV was first identified by Lockhart (1986) in Morocco. Its distribution is in Africa, Asia, Central and South America, and Oceania (Jones, 2000). The disease develops necrotic streaks on the midrib and petiole of banana leaves (Fig. 4A). Reduction of plant height, fruit malformation and size reduction, and



**Fig. 4.** Symptoms of BSV in Indonesia (A) and its vector *Planococcus citri* (B) (Photo B by Dr. Gede Suastika).

plant collapse have been reported. Some mealybug species (*Planococcus citri* and *Pseudococcus* spp.) are vectors of BSV in a semi-persistent manner (Fig. 4B).

At early stages of research, BSV was believed to be a single virus species with many strains and isolates from different continents. By comparison of their properties, Geering et al. (2000) and others found that they were very diversified. Recently, three BSV isolates were classified as independent member species of the genus *Badnavirus*.



**Fig. 5.** BSV transmission and BSV in host cells.

They are *Banana streak GF virus* (BSGFV), *Banana streak OL virus* (BSOLV) and *Banana streak Mysore virus* (BSMyV) (Fauquet et al., 2005). Though the name "Banana streak virus" is no longer used, for the purposes of this mini-review we will refer to these species collectively as Banana streak virus (BSV). The feature of diversified BSV is also well documented by Remans et al. (2007).

**Endogenous badnaviruses.** There are cases of unexpected BSV infection in tissue-cultured banana obtained from virus-free banana. This phenomenon was explained by the existence of diversified BSV sequences in the banana genome and their activation into intact BSV (Geering et al., 2005; Ndowora et al., 1999). Endogenous BSV sequences are partial and rearranged sequences with various numbers of copies and are integrated into banana genome. Replication of BSV can occur without this integration process. Under certain conditions, episomal BSV may arise in plant cells from endogenous BSV and develop symptoms on bananas (Fig. 5). Such integration of virus sequences into the host plant genome has been found in the genus *Geminivirus* with ssDNA and, as they use reverse transcription for genome replication, they are grouped as retroviruses. Pararetroviruses are another group of viruses with dsDNA found in the family *Caulimoviridae*, and the sequences that integrate into the host genome are designated as endogenous pararetroviruses (EPRVs). In addition to BSV, EPRVs for some species in the *Caulimoviridae* include *Tobacco vein clearing virus* in the tobacco genome, *Petunia vein clearing virus* in the petunia genome and *Rice tungro bacilliform virus* in the rice genome. The function of their integration remains unclear (Staginnus and Richert-Poggeler, 2006). Activation triggers of EPRVs are low temperature, low lighting, tissue culture and other stress factors on plants. In breeding programs of bananas and other crops EPRVs are believed to be a serious problem.

**Detection and protection of BSV.** The difficulty in detecting BSVs is due to their genetic and serological diversity. As for ELISA, an antibody to *Sugarcane bacilliform virus* (SCBV) in the genus *Badnavirus* is commercially available. Although SCBV is distinct from BSV, it is transmitted to banana, shows banana streak symptoms experimentally, and has serological relationships with some isolates of BSVs. However, the molecular and serological divergence of BSVs means that there is a need for the development of both badnavirus-universal and badnavirus species- and/or strain-specific detection technologies. The endogenous BSV makes episomal BSV detection more difficult. The combination of serological and molecular technologies such as immuno-capture PCR can discriminate episomal and endogenous infection. The control of

BSV relies on eradication of infected bananas and vectors, strict plant quarantine, as well as the use of endogenous BSV-free bananas as propagation materials.

**Future study on badnaviruses.** Until recently, the study on badnaviruses in Asia has been focused on limited virus species with economic importance. The discovery of EPRVs, however, needs further study. In particular, research is required on the distribution of episomal and endogenous badnaviruses in other hosts, the mechanism of integration and activation, the influence on plant resistance reactions through induction of gene silencing, and technologies to minimize the risks in plant breeding. The study of the genus *Badnavirus* can give insights into evolutionary interactions between viruses and host plants.

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