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VIRAL AND VIROID DISEASES

First report of *Bean common mosaic virus* in yam bean [*Pachyrhizus erosus* (L.) Urban] in Indonesia

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Abstract Severe mosaic with leaf malformation and green vein banding was observed on yam bean in West and Central Java, Indonesia. Virions of the causal virus were flexuous filaments, about 700 nm in length, with a coat protein of 30 kDa. The virus was transmitted by mechanical inoculation and by aphids in a nonpersistent manner. The nucleotide sequence of the coat protein gene had the highest identity with that of *Bean common mosaic virus* (BCMV, genus *Potyvirus*) isolate VN/BB2-5. Based on demarcation criteria, including the genome sequence and host range, we tentatively designate this isolate as BCMV-IYbn (Indonesian yam bean).

Keywords Nucleotide sequence · *Bean common mosaic virus* · *Pachyrhizus erosus* · *Potyvirus*

Yam bean [*Pachyrhizus erosus* (L.) Urban, Fabaceae], a horticultural crop in several areas of Indonesia, is used in Indonesia as fruit salads and cosmetic materials. The seeds and leaves are also used as botanical insecticides. Besides these beneficial roles, the yam bean is a symbiont with nitrogen-fixing bacteria and can therefore act as a good source of nitrogen in the soil (Sorensen 1996). In June 2004, viral-disease-like symptoms were observed during a survey of many yam bean fields in Bogor, West Java, and Prembun, Central Java, in Indonesia. Mosaic symptoms with green vein banding and leaf malformation were frequently observed (Fig. 1), affecting 14–100% of the plants in Bogor and 20–100% in Prembun. A poty-like virus was inferred to be the possible cause of the viral-disease-like symptoms. Previously, Sorensen (1996) reported that a potyvirus, *Bean common mosaic virus* (BCMV) might become a serious problem locally on cultivated yam bean in Tonga, Costa Rica, Ecuador and Thailand. However, Sorenson (1996) did not describe the biological or molecular characteristics of the BCMV infecting yam bean.

Since we had failed to find local lesion hosts to isolate the virus in preliminary studies, we first diagnosed a diseased plant serologically. Hereafter in this study, the virus source in all experiments except for some seed transmission tests was an infected single yam bean plant from a field in Bogor. The sap from the plant was serologically tested in an enzyme-linked immunosorbent assay (ELISA) using several antisera against viruses infecting legumes such as *Cucumber mosaic virus* (AS-0475; DSMZ, German Resource Center for Biological Material, Braunschweig, Germany), *Cowpea aphid-borne mosaic virus* (AS-0417; DSMZ), BCMV (AS-0159; DSMZ), *Soybean mosaic virus* (AS-0543; DSMZ) and *Bean yellow mosaic virus* (AS-0471; DSMZ) and nonlegume-infecting potyviruses such as *Chili veinal mottle virus* (AS-0122; DSMZ), *Turnip mosaic virus* (AS-0132; DSMZ), *Papaya ringspot virus* (PRSV, CAB-53500; Agdia, Elkhart, IN, USA) and *Watermelon mosaic virus* (WMV [synonyms Watermelon mosaic virus 2], CAB-54000; Agdia) and against the

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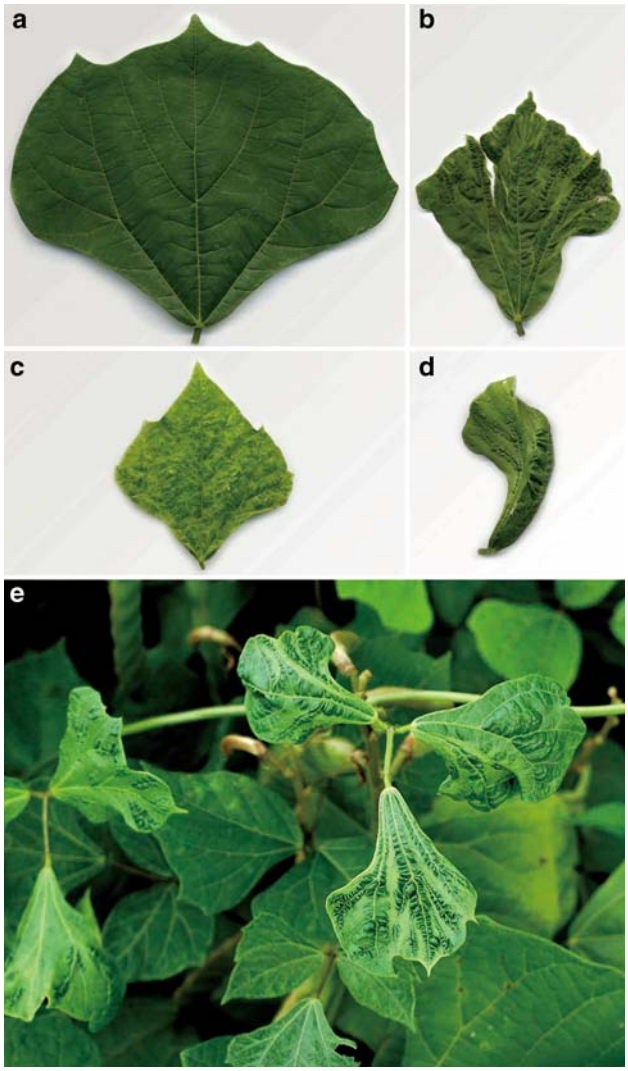

Fig. 1 Symptoms on yam bean leaves infected with *Bean common mosaic virus*-IYbn. **a** Healthy leaf. **b–d** Infected leaves. **b** Malformed leaf with green vein banding. **c**, Mosaic. **d** Leaf distortion. **e** Symptoms on yam bean plants in the field

tobamovirus *Tobacco mosaic virus* (AS-0041; DSMZ) and the comovirus *Squash mosaic virus* (CAB-26400; Agdia). We also used anti-potyvirus antiserum (AS-573/1 DSMZ). The sap was positive for anti-WMV2 (CAB-54000) and anti-potyvirus (AS-573/1) antisera, but negative for the other antisera. In a preliminary host range test using WMV2 indicator plants such as three species of cucurbits (listed in Table 1), *Chenopodium amaranticolor*, and *C. quinoa*, the virus did not infect those plants, suggesting that the causal agent of this mosaic disease in yam bean is a potyvirus apart from the ones we tested. Supporting this observation, WMV2 is serologically related to most of the mosaic-inducing and necrotic-inducing strains of BCMV (Edwardson 1974; Morales and Bos 1988).

Table 1 Host range of the virus isolated from yam bean, host symptoms after mechanical inoculation and results of ELISA

Plant family/species	Symptoms	ELISA[a]
Amaranthaceae		
Gomphrena globosa	−/−	+
Chenopodiaceae		
Chenopodium amaranticolor	−/−	−
C. quinoa	−/−	−
Compositae		
Ageratum conyzoides	−/−	−
Cruciferaceae		
Brassica olearacea	−/−	−
Cucurbitaceae		
Cucumis sativus cv. Venus	−/−	−
C. melo cv. Honey dew	−/−	−
Cucurbita maxima	−/−	−
Leguminosae		
Phaseolus vulgaris	−/M	+
Vigna sinensis	−/C	+
V. unguiculata	−/−	−
Arachis hypogaea cv. Gajah	−/−	−
Pisum sativum	−/MM	+
Solanaceae		
Nicotiana tabacum cv. White Burley	−/−	−
Lycopersicon esculentum cv. San Marino	C/(C)	(+)
Capsicum annuum cv. Bara	−/−	−
Datura stramonium	−/−	−
Physalis floridana	−/−	−

On inoculated/upper leaves. *C* chlorosis, *M* mosaic, *MM* mild mosaic, − no symptoms, parentheses indicate occasional infection

[a] Upper leaf tissues were assayed 1 month after inoculation. ELISA data were considered to be positive if the absorbance values of the tests were more than twice those of healthy plants

After these serological tests, infected plant tissue was further used to inoculate a number of healthy yam bean plants. The infected tissues were also used for virion purification for electron microscopy and protein analysis, as an inoculum source for aphid transmission and host range tests, and for the extraction of total RNA for nucleotide sequencing of RT-PCR products unless otherwise stated.

The morphology and size of the viral particles were examined by transmission electron microscopic (JEM 1010 JEOL, Tokyo, Japan) analysis of the purified virus after purification from infected leaves with the method of Berger and Shiel (1998). Electron microscopy confirmed the presence of potyvirus-like particles, with a flexuous filamentous morphology and about 700 nm in length (Fig. 2a). The purified virus was dissociated and subjected to sodium dodecyl sulfate-polyacrylamide gel

(12.5%) electrophoresis according to Laemmli (1970) and stained with Coomassie brilliant blue. The purified virus preparation produced a band of approximately 30 kDa and a smaller protein band, which could have been a degradation product of the full-length coat protein, as observed for many potyviruses (Fig. 2b). The virus preparation was then used to inoculate healthy yam bean plants, which led to mosaic symptoms on the plants, which were identical to the original symptoms on the field plants and the initial infected plant (data not shown). We tentatively designated this virus isolate as BCMV-IYbn (Indonesian yam bean) because of the additional characteristics described next.

Mechanical transmission was examined by inoculating healthy yam bean plants with the sap of infected yam bean plants. Aphid transmission was also tested with the following methods. Aphids (*Aphis craccivora* Koch, *A. glycines* Matsumura, and *A. gossypii* Glover) were identified using the identification key of Blackman and Eastop (2000). The aphids were starved for 1 h and allowed to feed for either 3 min or overnight on the diseased plants. After both periods of acquisition feeding, the viral symptoms were reproduced after the first inoculation feeding (30 min), but not after second and third feedings. These results indicated that the virus was efficiently transmitted mechanically and by all aphids tested in a nonpersistent manner. In a test of the efficiency of aphid transmission on ten yam bean plants for each aphid species, up to 100% of tested plants showed symptoms with transmission by *A. craccivora* and *A. gossypii* and 70% of those by *A. glycines*. The virus was reisolated from all symptomatic plants, confirmed either by ELISA as already described or RT-PCR as described later.

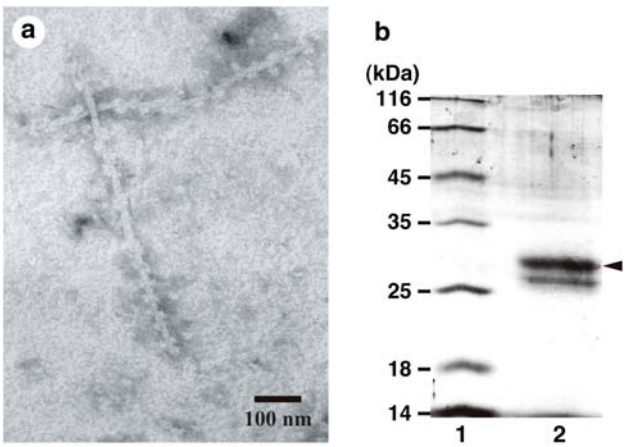

Fig. 2 **a** Electron micrograph of purified virus particles negatively stained with 2% uranyl acetate. *Bar* 100 nm. **b** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of coat protein. Lane *1*, Unstained protein molecular-weight marker; lane *2*, *Bean common mosaic virus*-IYbn (*arrowhead* indicates the coat protein of 30 kDa)

In seed transmission tests with two seed lots, one seed lot was harvested from the inoculum source obtained as described, and the other seed lot was from infected yam bean plants grown in the fields in Bogor. Each seed lot (100 seeds) was divided into ten composite samples of ten seeds each. Fourteen days after seeding, leaves were harvested and subjected to ELISA as described. In the first seed lot, one of ten composite samples was positive, while two of ten in the last seed lot were positive for BCMV-IYbn using anti-potyvirus antiserum (AS-573/1) (data not shown). These results suggest that BCMV-IYbn could be transmitted through seeds.

Infected plants with typical severe mosaic symptoms and leaf malformations were used for mechanical inoculation with 0.1 M phosphate buffer (pH 7.2). The host range for the virus was examined in a mechanical inoculation assay of plants belonging to 18 species from seven families. The inoculated plants were maintained for 4 weeks in a greenhouse until the typical symptoms appeared. In all plants assayed, the virus was detected serologically in the upper leaves with ELISA using the anti-potyvirus antiserum (AS-573/1). The host range and symptomatology of the virus are presented in Table 1. Thirteen species from six families were not infected at all by this virus, whereas five species from three families were systemically infected. Systemic infection occurred in *Phaseolus vulgaris*, *Vigna sinensis*, *Pisum sativum*, and occasionally in *Lycopersicon esculentum* and asymptomatically in *Gomphrena globosa*. No local lesions were formed on the inoculated leaves of any plant species tested.

Total RNA was extracted from the infected yam bean plants with a total RNA extraction kit (Qiagen Sciences, Germantown, MD, USA). The cDNA was synthesized with SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) using the primer M4T (5′-GTTTTCCCAGTCACGACTTTTTTTTTTTTTT-3′) for the 3′ terminal poly(A) sequences of potyviruses. The cDNA was amplified by PCR using M4 (5′-GTTTTCCCAGTCACGAC-3′) and S-primer (5′-GGNAAYAAYAGYGGNCARCC-3′; N = A, C, G, or T; Y = C or T; R = A or G), which was designed from the consensus sequence that encodes the conserved amino acid sequence GNNSGQP in the NIb region of the family *Potyviridae* (Chen et al. 2001). The 1.7-kb PCR products thus obtained were also detected when viral RNA extracted from purified virions was used as the template (data not shown). The PCR products were separated on 1% SeaPlaque GTG agarose gels (Cambrex, Rockland, ME, USA) and purified using Wizard SV gel and PCR Clean-up System (Promega, Madison, WI, USA). The gel-purified DNA was directly subjected to nucleotide sequencing with an ABI 310 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Additional appropriate primers were designed to sequence the coat protein (CP)

gene and the 3′ untranslated region (UTR) of the viral genome.

The nucleotide sequences of the CP gene and the 3′ UTR of the viral genome were aligned with those of other potyviruses using the program Clustal W (Thompson et al. 1994), while sequence identities were calculated using "sequence identity matrix" option in the program BioEdit version 7.05 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The phylogenetic tree was constructed from the Clustal W-aligned sequences using the program MEGA 4.0 (Tamura et al. 2007) with the neighbor-joining method and Kimura 2-parameter model to estimate the distances, and bootstrap support was estimated with 1,000 replicates. The nucleotide sequences of both regions of the virus had the highest identities with those of BCMV isolate VN/BB2-5 (DQ925422), which is reported to infect *Phaseolus vulgaris* (black bean) in Vietnam (Ha et al. 2008) (86.5% identity to the CP nucleotide sequence, 91.6% to the CP amino acid sequence, and 92.9% to the 3′ UTR sequence). The identities of the CP gene and the 3′ UTR of the virus relative to those of other potyviruses were 59.2–74.0% and 27.7–78.8%, respectively (Table 2). According to Shukla and Ward (1989), the nucleotide sequence identity of the CP gene among strains of a certain potyvirus species was over 90%, whereas Frenkel et al. (1989) reported that the degree of identity in the nucleotide sequences of the 3′ UTR between strains of certain potyvirus species was 83–99% and distinct virus species have identities in the range of 39–53%. Adams et al. (2005) reviewed numerous sequences for potyviruses and proposed that the demarcation for the optimal CP nucleotide sequence identity was 76–77% and that for the CP amino acid sequence identity was 82% in the same potyvirus species. Both the nucleotide and amino acid sequences of the CP gene and the 3′ UTR sequence were highly homologous to those of the BCMV strains (Table 2), especially BCMV isolate VN/BB2-5. The identities of the nucleotide sequences of the CP gene and the 3′ UTR and the amino acid sequences of the CP of the yam-bean-infecting virus and BCMV isolate VN/BB2-5 were much higher than 77, 83, and 82%, respectively.

In phylogenetic analyses based on the CP-nucleotide sequences, isolates of BCMV were grouped into two major clusters (Fig. 3). Isolates IYbn and VN/BB2-5 were in the same cluster, while other BCMV strains, represented by the peanut stripe (PSt) strain and the blackeye cowpea (BlC) strain, were grouped into another cluster. Based on the identities for the CP-coding region, VN/BB2-5 was distantly related to other viruses of the BCMV group (Ha et al. 2008). In addition, an ELISA of BCMV-IYbn showed that BCMV-IYbn reacted negatively to anti-BCMV-PSt antiserum (AS-0159; DSMZ) (data not shown). These results suggest that BCMV-IYbn as well as BCMV-VN/BB2-5 is distantly related to other BCMV strains and could be designated as a new potyvirus species based on the criteria by Adams et al. (2005). However, they also stated that for potyvirus species identification, the CI gene, rather than the CP gene, is the best gene to determine potyvirus species. Therefore, determination of the complete sequences of these isolates is required to clarify their relationships to

Table 2 Percentage[a] sequence identity between the coat protein gene and the 3′ untranslated region (3′ UTR) of the virus isolated from yam bean[b] and those of other potyviruses

Virus[c]	Coat protein[d]		3′ UTR	GenBank accession
	Nt	aa		
AzMV	71.1	73.1	47.8	U60100
BCMV-BlC-R	72.1	74.9	78.8	AJ312437
BCMV-BlC-VN/YB1	72.1	74.9	77.6	DQ925424
BCMV-PSt	74.0	76.3	75.0	X63559
BCMV-PSt-I14	73.8	76.6	63.1	AJ132157
BCMV-PSt-VN/SB1	73.7	76.3	74.2	DQ925418
BCMV-VN/BB2-5	86.5	91.6	92.9	DQ925422
BYMV	59.2	55.1	27.7	NC_003492
CabMV	66.5	67.8	42.6	NC_004013
SMV	69.6	75.0	59.2	AY294044
WMV	69.7	72.2	58.2	AB218280

[a] Sequence identities were calculated using "sequence identities matrix" option in BioEdit version 7.05

[b] Accession number of BCMV-IYbn is AB289438

[c] *AzMV* Azuki bean mosaic virus (a member of BCMV), *BCMV Bean common mosaic virus*, *BCMV-BlC* blackeye cowpea strain, *BCMV-PSt* peanut stripe strain, *BCMV-VN/BB2-5* BCMV isolate VN/BB2-5, *BYMV Bean yellow mosaic virus*, *CabMV Cowpea aphid-borne mosaic virus*, *SMV Soybean mosaic virus*, *WMV Watermelon mosaic virus*

[d] *Nt* nucleotide, *aa* amino acid

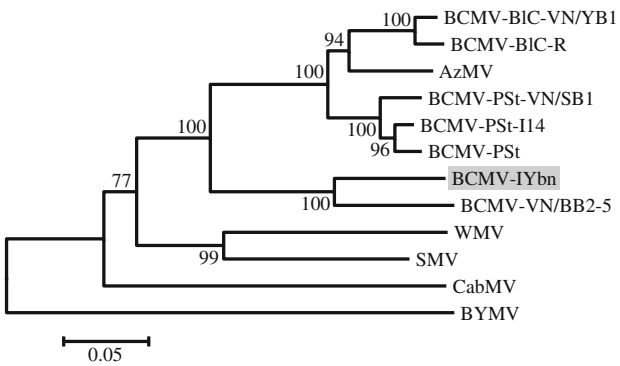

Fig. 3 A phylogenetic tree based on the nucleotide sequences of the coat protein genes of the seven *Bean common mosaic virus* (BCMV) isolates and other potyviruses. The numbers at the branch node indicate the confidence values (%) from bootstrap analysis using 1,000 replicates. Outgroup: *Bean yellow mosaic virus* (BYMV). See Table 2 for the GenBank accession numbers of the viruses. BCMV-IYbn is highlighted

other BCMV isolates as noted for BCMV-VN/BB2-5 (Ha et al. 2008).

Experimental hosts for BCMV are predominantly legumes, although *Arachis hypogaea* and *Pisum sativum* are reported to be nonhosts (Morales and Bos 1988). Our findings differ somewhat from the general BCMV host ranges. The BCMV isolated from yam bean was able to infect *P. sativum*, occasionally induced systemic chlorosis in *L. esculentum*, and latently infected *G. globosa*. This report is the first on the occurrence of BCMV in yam bean, which causes severe mosaic, in Indonesia, and we named this isolate BCMV-IYbn (Indonesian yam bean).

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