Research

Incidence of Bamboo Mosaic Potexvirus in Taiwan

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ABSTRACT

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Bamboo mosaic virus (BaMV) was immunologically detected by direct tissue blotting in 11 bamboo species, including three varieties and three cultivars, in Taiwan. Viral RNA was detected in one additional bamboo species, *Melocanna baccifera*, by hybridization with a specific riboprobe for BaMV RNA. Most infected bamboo species have pachymorph rhizomes and belong to the genera *Bambusa* and *Dendrocalamus*. Species with other types of rhizomes were only rarely infected.

Additional keywords: diagnosis, disease indexing method

The subfamily Bambusoideae contains 57 genera and more than 1,200 species that are distributed all over the world. Of these, 46 species in 16 genera are known to grow naturally or are cultivated in Taiwan (10). In a preliminary survey made in 1979 by Lin et al (7), five bamboo species, including two varieties and one cultivar, were infected with bamboo mosaic virus (BaMV) (2). The virus causes a mosaic symptom and reduced vigor in the infected plants. Since bamboo is normally vegetatively propagated and the virus is mechanically transmissible, the practice of routine mechanical cutting aids the spread of the pathogen. Bamboo mosaic is considered a threat to the bamboo industry in Taiwan.

BaMV has a flexuous, rod-shaped morphology (3,5) and a genome of single-stranded, positive-sense RNA about 6.4 kb in length (9). The virus is a member of the potexvirus group (3,9). Recently, we successfully used the direct tissue blotting technique to detect the BaMV antigen in infected bamboo plants (8). The antigen was easily detected by immunostaining tissue blots prepared from infected leaves, petioles, sheath blades, and newly emerged shoots.

Since the 1979 survey (7), the disease has spread in Taiwan, and a greater number of bamboo species have been found to be infected. In this study, we used tissue-blotting techniques to detect the BaMV antigen and BaMV RNA in bamboo.

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MATERIALS AND METHODS

Virus isolation and purification, and RNA extraction. BaMV was isolated from infected green bamboo (*Bambusa oldhamii* Munro) (6). Procedures for virus purification and RNA extraction were described previously (6,9).

Tissue blotting on nitrocellulose (NC) membrane. Newly unrolled bamboo leaves were collected at Taipei, Chiayi, and Kaoshiung, from bamboo nurseries maintained by the Taiwan Forest Research Institute. The leaves were cut with a new razor blade, and the tissue blots were obtained by pressing the cut surface onto NC membrane with a pore size of 0.45 μm (Schleicher & Schuell, Inc., Keene, NH) (8). For each sample, three duplicated blots were made on separate NC sheets; and for each cut surface, two prints were produced. Two sheets were used for the immunological detection of the BaMV antigen, and another was used for the detection of BaMV RNA by hybridization.

Preparation of immunoprobe for the detection of viral antigen. Procedures for the preparation of rabbit anti-BaMV capsid protein serum (anti-BaMV-CP serum) were described previously (6). Purified BaMV was disrupted in a protein-sample buffer and electrophoresed in a 12% polyacrylamide gel. For visualization of the protein band, the gel was incubated in 0.25 M KCl at 4 C for 30 min. The BaMV capsid protein band corresponding to the portion of M_r 28,000 was excised, and the protein was eluted with an ISCO protein concentrator. Antiserum to the gel-eluted BaMV

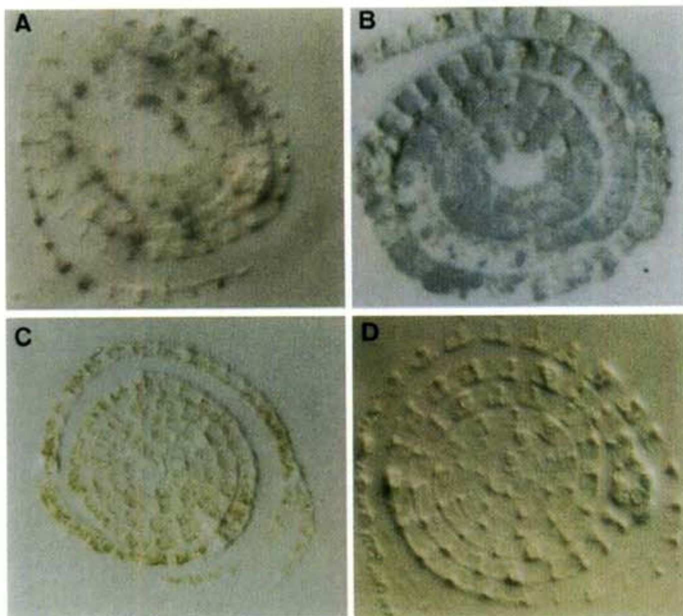

Fig. 1. Dissecting micrographs of leaf-tissue blots on nitrocellulose membrane showing immunological detection of the bamboo mosaic virus (BaMV) antigen. The blots were reacted with diluted anti-BaMV-capsid protein serum **(A–C)** or preimmune serum **(D)** and detected with alkaline phosphatase conjugated goat anti-rabbit IgG. The leaf blots were made from bamboo species *Dendrocalamus latiflorus* **(A)**, *Bambusa vulgaris* **(B,D)**, and uninfected *D. latiflorus* **(C)**.

capsid protein was prepared in New Zealand white rabbits by four consecutive intramuscular injections with 1 mg/ml of purified capsid protein.

Detection of the BaMV antigen on NC membrane. The following indirect immunological procedure was performed (8). After blocking, the NC sheet was incubated with a 1:1,000 dilution of rabbit anti-BaMV-CP serum (6) or preimmune serum. This was followed by reaction with alkaline phosphatase conjugated goat anti-rabbit IgG (Sigma, St. Louis, MO 63178). A combination of nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) was used as a substrate for color development. The results were photographed under a dissecting microscope with an optical magnification of 20X.

Preparation of in vitro ^{32}P-labeled riboprobe. DNA complementary to BaMV RNA was synthesized by priming with oligo(dT), then cloned into vector pUEX1 (Amersham International, Amersham, Buckinghamshire, England). A cDNA clone, pBaHB, which covered the 3′ terminal 173 nucleotides of BaMV RNA, was selected and subcloned into pGEM4 transcription vector (Promega Corp., Madison, WI 53711) for the in vitro transcription reaction. The in vitro ^{32}P-labeled RNA transcript was synthesized from *Hin*dIII-linearized pBaHB in the presence of [α-^{32}P]CTP and used as a hybridization probe to detect positive-strand viral RNA (4). In the transcription reaction (25 μl), 1 μg of linearized template DNA was added to 5 μl of 5X transcription buffer (Promega); 2.5 μl of 0.1 M DTT; a 5-μl mixture of 5-mM ATP, GTP, and UTP, and 100-μM CTP; 0.5 μl of RNasin

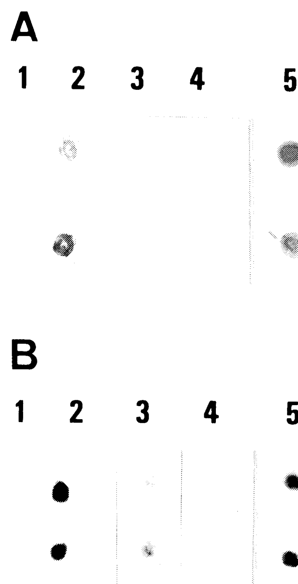

Fig. 2. Immunological and RNA hybridization assays for bamboo mosaic potexvirus (BaMV) in bamboo leaf blots on nitrocellulose membrane. Duplicated prints were made for each sample. **(A)** Blots were reacted with diluted anti-BaMV-capsid protein serum and detected with alkaline phosphatase conjugated goat anti-rabbit IgG. Column 1 = *Pseudosasa japonica* showing mosaic-like symptoms, column 2 = *Dendrocalamus dolichoclada* 'Stripe' showing mosaic symptoms, column 3 = symptomless *Melocanna baccifera,* and column 4 = healthy-looking *D. dolichoclada* 'Stripe'. Column 5 = 0.1 μg of BaMV virion dotted as a control. **(B)** Blots were pretreated with a 0.5% sodium dodecyl sulfate (SDS) solution containing 100 μg/ml proteinase K and hybridized with BaMV RNA-specific ^{32}P-labeled riboprobe. Columns 1–4 were made from the same plants as those in **(A)**. Column 5 = 50 pg of BaMV RNA blotted as a control. Columns 1–4 were exposed to X-ray film for one week at -70 C with an intensifying screen, while column 5 was exposed overnight only.

Table 1. Incidence of bamboo mosaic virus (BaMV) infection in Taiwan as determined by symptomatology, and by immunological (IM) and RNA hybridization (HB) assays of tissue blots

Bamboo species	Mosaic symptoms[a]	IM assays[b]	HB assays[c]
Arthrostylidium naibunensis Lin	−	−	−
Arundinaria graminea Makino	−	−	−
A. hindsii Munro	+	−	−
A. linearis Hackel	−	−	−
A. simonii Rivière & C. Rivière	−	−	−
A. variegata Makino	−	−	−
Bambusa beecheyana Munro	+	+	+
B. beecheyana var. *pubescens* Lin	+	+	+
B. dolichoclada Hayata	+	+	+
B. dolichoclada Lin 'Stripe'	+	+	+
B. dolichomerithalla Hayata	−	−	−
B. dolichomerithalla Lin 'Green stripestem'	−	−	−
B. dolichomerithalla Lin 'Silverstripe'	−	−	−
B. edulis Keng	+	+	+
B. fecunda McClure	−	−	−
B. multiplex (Lour.) Raeusch.	−	−	−
B. multiplex Young 'Alphonse Karr'	+	+	+
B. multiplex Young 'Fernleaf'	−	−	−
B. multiplex Young 'Stripestem'	−	−	−
B. oldhamii Munro	+	+	+
B. pachinensis Hayata	+	+	+
B. pachinensis var. *hirsutissima* Lin	+	+	+
B. stenostachya Hackel	−	−	−
B. stenostachya Lin 'Wei-fang Lin'	−	−	−
B. tulda Roxb.	−	−	−
B. utilis Lin	+	+	+
B. variegata Siebold	−	−	−
B. ventricosa McClure	+	+	+
B. vulgaris Schrad. ex Wendl.	+	+	+
B. vulgaris var. *striata* Gamble	+	+	+
B. vulgaris McClure 'Wamin'	−	−	−
Chimonobambusa quadrangularis Makino	−	−	−
Dendrocalamus asper (Schultes) Heyne	−	−	−
D. giganteus Munro	+	+	+
D. latiflorus Munro	+	+	+
D. latiflorus Lin 'Mei-nung'	+	+	+
D. latiflorus Lin 'Subconvex'	+	+	+
D. strictus (Roxb.) Nees	−	−	−
Gigantochloa apus (Schultes) Munro	−	−	−
G. levis Merrill	−	−	−
G. verticillata Munro	−	−	−
Guadua angustifolia Kunth	−	−	−
Melocanna baccifera (Roxb.) Kurz	−	−	+
Ochlandra capitata E.G. Camus	−	−	−
Phyllostachys aurea Rivière & C. Rivière	+	−	−
P. bambusoides Siebold & Zucc.	+	−	−
P. lithophila Hayata	−	−	−
P. makinoi Hayata	−	−	−
P. nigra (Lodd.) Munro	+	−	−
P. nuda McClure	−	−	−
P. pubescens Mazel ex Houz.	−	−	−
Pseudosasa japonica (Steudel) Nakai	+	−	−
P. usawai Makino & Nemoto	−	−	−
Schizostachyum diffusum Merrill	−	−	−
Semiarundinaria fastuosa Makino	−	−	−
Shibataea kumasaca (Steudel) Nakai	−	−	−
Sinobambusa kunishii Nakai	−	−	−
S. tootsik Makino	+	−	−
Thyrsostachys siamensis Gamble	−	−	−

[a] + = mosaic symptoms; − = lack of mosaic symptoms.
[b] + = BaMV antigen detected; − = no BaMV antigen detected.
[c] + = BaMV RNA detected; − = no BaMV RNA detected.

(40 units per microliter); 2 μl of SP6 RNA polymerase (20 units per microliter); 4 μl of ribonuclease-free water; and 5 μl of [α-^{32}P]CTP (20 mCi/ml, 3,000 Ci/mmol, Amersham). The reaction was performed at 37 C for 60 min, and the labeled transcripts were purified with the spun-column procedure (11). Typically, the reaction yielded 10^8 cpm of radioactive RNA.

Detection of BaMV RNA on NC membrane. For the detection of BaMV RNA, the tissue blot was first soaked for 10 min in a 0.5% sodium dodecyl sulfate (SDS) solution containing 100 μg/ml of proteinase K. After air-drying, the sheet was baked at 80 C for 2 hr in a vacuum oven. Prehybridization and hybridization conditions followed the description by Kroner et al (4) for northern analysis. The hybridization mixture usually contained a 5×10^7 cpm radioactive riboprobe.

RESULTS

Immunological detection of the BaMV antigen by tissue blotting. The BaMV antigen was readily detected in infected leaf blots on NC membrane by its purplish color, whereas there was no color development in blots treated with diluted preimmune serum. Generally, an uneven distribution of BaMV antigen was observed when the blots were examined under a dissecting microscope (Fig. 1A). However, occasionally BaMV antigen was distributed over all of the blots (Fig. 1B). No viral antigen could be detected in blots prepared from uninfected leaves or in the healthy control blots. When viewed with a dissecting microscope, uninfected leaf blots (Fig. 1C) and infected leaf blots treated with preimmune serum (Fig. 1D) normally had the green color of chlorophyll.

The results of several independent experiments are summarized in Table 1. Among the 46 bamboo species in 16 genera that were tested (including three varieties and 10 cultivars), nine species (including three varieties and one cultivar) in the genus *Bambusa* and two species (including two cultivars) in the genus *Dendrocalamus* were found by immunotissue blotting to be infected with BaMV. Although some species in the genera *Phyllostachys, Pseudosasa,* and *Sinobambusa* showed mosaic-like symptoms on their leaves, no BaMV antigen was detected (Table 1; Fig. 2A, column 1).

Detection of BaMV RNA by tissue-blotting hybridization. To examine the specificity of the radioactive riboprobe, dot hybridization was performed using RNAs extracted from purified virus. As shown in Fig. 2B (column 5), 50 pg of BaMV RNA could be easily detected after overnight exposure. Detection of 5 pg of RNA with the ^{32}P-labeled riboprobe required an exposure time of 1 wk at -70 C with an intensifying screen (*unpublished*).

Viral RNA was detected by RNA hybridization in all bamboo species showing a positive reaction for the BaMV antigen. RNA hybridization also detected viral RNA in one species (*Melocanna baccifera* (Roxb.) Kurz) collected from localities where typical mosaic symptoms were not found, and for which no viral antigen was detectable by tissue blotting (Table 1; Fig. 2B, column 3). Viral RNA was detected by hybridization with a specific riboprobe after a 1-wk exposure (Fig. 2B), indicating a very low level of viral RNA in the leaves.

DISCUSSION

The incidence of bamboo mosaic in Taiwan was examined by immunological and RNA hybridization assays. The riboprobe hybridization was more sensitive than was the immunological assay, as evidenced by the detection of BaMV RNA, but not of the BaMV antigen, in *M. baccifera* (Table 1, Fig. 2). These results were consistent with those of Abad and Moyer (1), who found that the riboprobe system provides a greater sensitivity for the detection of sweetpotato feathery mottle virus than does the immunological assay by direct blotting on NC membrane.

Of the 46 bamboo species assayed, 12 (including three varieties and three cultivars) were found to be infected with BaMV. The disease incidence in Taiwan is higher now than in 1979 (7); however, the number of bamboo species showing mosaic symptoms was greater than the number showing a positive reaction in either hybridization or immunotissue blotting (Table 1). Those plants negative by immunological and RNA hybridization assays might have been infected with another virus or viruses which are unrelated to BaMV. Because the survey for BaMV incidence in 1979 was based primarily on symptoms and an infectivity test (7), discrepancies between the results of the 1979 and the 1991–1992 assays were not totally unexpected. For instance, the BaMV antigen and BaMV RNA were not detected by leaf blotting in *Phyllostachys nigra* (Lodd.) Munro, which showed mosaic symptoms. This was true with several other *Phyllostachys* species (Table 1).

In our survey, BaMV primarily infected bamboo species with rhizomes of the pachymorph type. However, *M. baccifera,* which has rhizomes of metamorph type II, was also infected. BaMV has not been detected in bamboo species with rhizomes of the leptomorph or metamorph type I. These results agreed with the reported disease incidence in Brazil, where both *B. multiplex* (Lour.) Raeusch. and *B. vulgaris* Schrad. ex Wendl. are infected with BaMV (5). Although susceptibility to BaMV was found primarily in bamboo species having pachymorph rhizomes, we cannot rule out the possibility that bamboo species with rhizomes of other types may also be susceptible.

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