MUNGBEAN YELLOW MOSAIC VIRUS

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ABSTRACT

The whitefly-borne mungbean yellow mosaic virus (MYMV) observed in Thailand was transmitted by mechanical inoculation. The optimal incubation temperatures for symptom expression on plant ranged from 25 to 30°C in the growth chamber. Host range of MYMV was limited to seven plant species in the family Leguminosae. Determination of the stability of the virus in plant sap gave the following results : thermal inactivation point : 40-50°C for 10 min, dilution end point : 10^{-2}-10^{-3}, and longevity in vitro : 1-2 days at 20°C. Purified virus preparations consisted of geminate particles about 18 × 30 nm in size with an ultraviolet light absorption spectrum typical of that of nucleoprotein (A_{260}/A_{280} value of 1.3-1.4). Infectivity was associated with the presence of purified virus particles. In ultrathin sections, the virus particles were isometric, about 15-20 nm in diameter, and aggregates of virus particles sometimes filled almost completely the nuclei of infected phloem cells as early as 2 days before symptom appearance. Nucleic acid isolated from MYMV particles was identified as circular single-stranded DNA with a molecular weight of 8.0×10^5. MYMV was assigned to the Geminivirus group.

Introduction

In 1977, the first report on the occurrence of mungbean yellow mosaic disease outbreak in Thailand was received from Kamphaeng Phet Province in northern Thailand (Thongmeearkom *et al.*, 1981). The disease also occurred in five other nearby provinces and caused almost total yield loss in infected mungbean (*Vigna radiata*) plants in the field. Laboratory tests indicated that the causal agent of the disease was transmitted by the tobacco whitefly, *Bemisia tabaci* Genn. (Thongmeearkom *et al.*, 1981). Prior to that report, a similar whitefly-borne disease had been observed in India (Nariani, 1960). The disease in India was very severe in blackgram (*V. mungo*) and caused as much as 100% yield loss when plants were infected at the seedling stage (Nene, 1973). Despite the severity of the disease in both countries, very little was known previously about the nature of the causal agent except that it was whitefly-borne and was not transmissible by mechanical inoculation (Nene, 1973). Isolation and characterization of the causal agent of mungbean yellow mosaic disease have not been reported.

In this paper, I report the mechanical transmission, purification and ultrastructural studies of mungbean yellow mosaic virus (MYMV) in Thailand and describe some of the properties indicating that MYMV is a member of the Geminiviruses.

Virus source and maintenace

Mungbean plants showing yellow mosaic symptoms were collected from fields in the Kamphaeng Phet Province of northern Thailand in 1979. The virus was isolated from naturally infected mungbean plants by whitefly (*B. tabaci*) transmission and maintained in mungbean plants by whitefly and grafting transmission in a greenhouse.

Mechanical inoculation

Seedlings of mungbean used for mechanical inoculation tests were 5-7 days old. All inocula were prepared by grinding infected young mungbean leaves in buffers (about 4 ml/g tissue) with

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a chilled mortar and pestle. Inoculations were made by rubbing Carborundum-dusted primary leaves of the test plants with cotton wool soaked in the homogenate.Various buffers (potassium phosphate, sodium phosphate, borate and Tris-HC1) with molarities of 0.05, 0.1 and 0.2 were used in the transmission studies. Among the buffers, 0.1 M potassium or sodium phosphate, pH 7.8, gave transmission rates as high as 90-100%. In potassium phosphate buffer at pH 4.5, 5.0, 6.0, 6.5, 7.0, 7.5, 7.8, 8.0, 8.5, 9.0, and 9.5, the percentages of mungbean seedlings with yellow mosaic symptoms were 25, 15, 50, 80, 100, 95, 100, 90, 90, 85, and 70%, respectively. The optimum pH values of phosphate buffer for transmission ranged between 7.0 and 7.8. At 15, 20, 25, 30, and 35°C in the growth chamber, the percentages of transmission were 0, 80, 87, 93, and 80%, respectively, and 97% in the greenhouse, where the temperature varied from 20°C minimum at night to 30°C maximum in the daytime. Although no symptoms appeared at 15°C, 70% of the test plants showed symptoms when maintained at 15°C for 23 days and then kept in the greenhouse for 22 days.

Host range and stability in sap

Host range of MYMV was determined by mechanical inoculation of 26 plant species in 6 families. Young seedlings were used in these trials and were inoculated under optimal conditions for MYMV transmission. Inoculated plants were assayed by back-inoculation to mungbean seedlings 24 days after inoculation. Only seven species of the family Leguminosae were infected with MYMV. Symptoms consisted mainly of yellow mosaic or leaf curl. In systemically infected leaves of azuki bean (*Phaseolus angularis*), blackgram (*V. mungo*), mungbean (*V. radiata*) and soybean (*Glycine max*), irregular chlorotic spots along the veinlets appeared at the early stages, then developed into severe yellow mosaic symptoms. First emerging trifoliolate leaves of mungbean showed severe downward curling. In infected bean (*P. vulgaris*), trifoliolate leaves showed downward curling without yellow mosaic symptoms. In infected jack bean (*Canavalia ensiformis*) and lima bean (*P. lunatus*), irregular chlorotic spots developed in leaflets, followed by mild downward curling. Back-inoculation to mungbean seedlings from infected plants resulted in yellow mosaic symptoms in test plants.

In sap extracted from infected mungbean leaves, the virus showed thermal inactivation point of 40-50°C for 10 min, dilution end point ranging between 10^{-2} and 10^{-3}, and longevity in vitro of 1-2 days at 20°C.

Virus purification

Systemically infected leaves of bean (*P. vulgaris* 'Top Crop') were homogenized with a Waring Blender in 0.1M potassium phosphate buffer, pH 7.8, containing 0.1% thioglycolic acid, 10 mM sodium diethyldithiocarbamate, and 1 mM sodium ethylenediaminetetraacetate (2 ml/g tissue). The extract was clarified by adding one-half volume of chloroform. The emulsion was broken by centrifugation after stirring at 4°C for 30 min. Polyethylene glycol (PEG: mol. wt. 6,000) and sodium chloride were added to the aqueous phase to give a final concentration of 6% and 0.2M, respectively. After stirring, the mixture was centrifuged at 15,000 *g* for 30 min and the precipitates were dissolved in 0.1 M potassium phosphate, pH 7.8, and clarified by low-speed centrifugation before being subjected to ultracentrifugation at 125,000 *g* for 90 min. The pellets were resuspended in potassium phosphate containing 6% PEG and 0.2 M NaCl. About 2 ml of the resuspended pellets were layered onto PEG discontinuous reverse solubility gradients and centrifuged in a Hitachi RPS 25 swinging rotor at 12,000 rpm for 20 min. The opaque band located at the interface between sucrose layers contining 4 and 0% PEG was recovered and concentrated by ultracentrifugation as before. Resuspended pellets were subjected to 10-40% linear sucrose density gradient ultracentrifugation. After centrifugation, gradients were analysed and fractionated by an ISCO Model 640 density gradient fractionator coupled with an ISCO Model

UA-5 absorbance monitor. A single band was located at fractions 9–15 (Fig. 1). When employed for mechanical inoculation, these fractions proved infective in 5 mungbean seedlings (Fig. 1). Symptoms obtained were similar to those shown by mungbean plants infected with MYMV. Ultraviolet light (254 nm)-absorbing fractions were concentrated by ultracentrifugation. The size of the purified geminate particles was about 18 = 30 nm (Fig. 2A, B). Electron microscopy of leaf-dip samples prepared from young mungbean leaf tissues infected with MYMV also revealed particles of similar size and shape (Fig. 2C). The corresponding fractions from healthy tissue treated similarly failed to show a peak and geminate particles. The preparations with geminate particles had an ultraviolet light absorption spectrum characteristic of that of nucleoprotein (A_{260}/A_{280} value of 1.3–1.4).

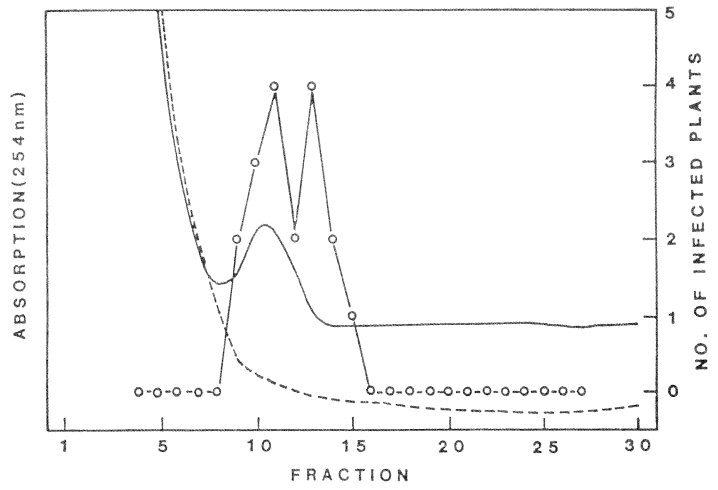

Fig. 1 Sedimentation profiles of purified extracts from mungbean yellow mosaic virus-infected Top Crop beans and healthy beans in 10–40% linear sucrose gradients and infectivity associated with the fractions collected. Sedimentation from the left. --- = Healthy leaf extract absorbance; o—o = infected leaf extract absorbance; and = infectivity (each fraction collected was inoculated to five mungbean seedlings).

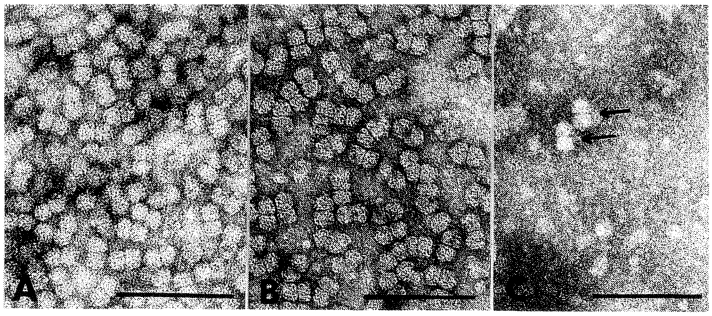

Fig. 2 Electron micrographs of mungbean yellow mosaic virus particles. (A and B) : Purified virus preparations negatively stained with 2% sodium phosphotungstate (PTA), pH 3.5, and 2% uranyl acetate, respectively. (C) : Infected mungbean leaf-dip samples negatively stained with 2% PTA, pH 3.5. Arrows indicate geminate particels. Bar = 100 nm.

Ultrathin sectioning

Mungbean leaf samples for ultrathin sectioning were collected from inoculated plants 5, 7, 9, 11, 13, 15, and 18 days after inoculation either by whitefly or by grafting. Pieces of the infected leaves were fixed with 4% glutaraldehyde at 5°C for 1.5 hr, and were post-fixed with 2% osmium tetroxide in 0.1 M phosphate buffer, pH 7.5, at 5°C for 5 hr. After washing and dehydration, they were embedded in a mixture of low-viscosity epoxy resin (Spurr, 1969). Ultrathin sections were cut with a glass knife in a LKB Ultrotome. They were double-stained with uranyl acetate and lead citrate before observation. Leaf samples from non inoculated mungbean plants at comparable age were similary processed and served as controls. The first ultrastructural changes observed were in phloem tissues sampled 7 days after inoculation (2 days prior to symptom appearance). The nucleoli of some phloem cells from such symptomless tissue were hypertrophied. Loose aggregates of MYMV particles were also observed in the nucleus. The aggregates varied in size and shape, and sometimes occupied almost the total nuclear volume (Fig. 3A, B). The aggregates of virus particles were more frequently observed in the nucleus as the time after inoculation progressed. These aggregates were found in nuclei regardless of the presence of the nucleoli. The diameter of the virus particles was 15-20 nm. Fibrillar bodies (usually one or two per nucleus) with the shape of either solid circles of rings, depending upon the orientation of sectioning, were occasionally observed along the edge of the aggregates of virus particles or scattered in the nucleoplasm.

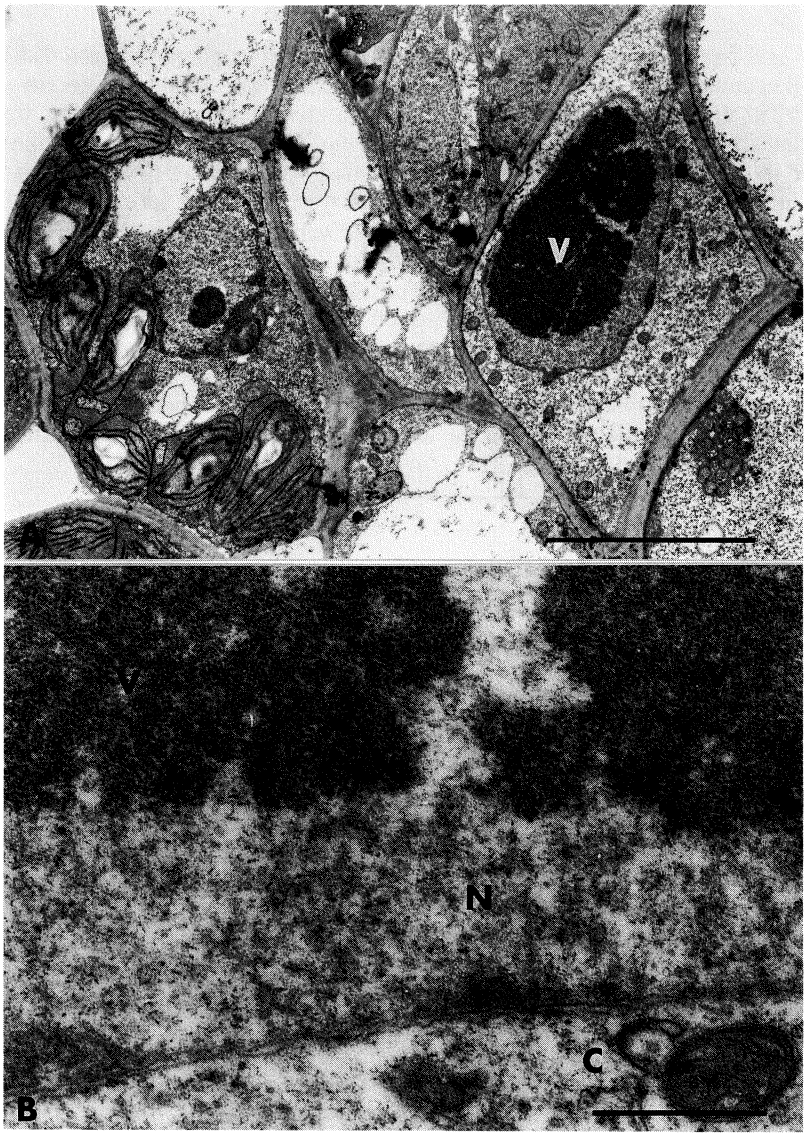

Fig. 3 Ultrastructure of mungbean yellow mosaic virus-infected mungbean leaf. (A) : The aggregate of virus particles (V) occupying almost the total nuclear volume of the phloem cell. Bar = 5 μm. (B) : Enlarged view of (A). Individual virus particles (V) can be seen in the nucleus (N). C = cytoplasm. Bar = 0.5 μm.

Nucleic acid of virus

Nucleic acid from purified MYMV particles was isolated by phenol-sodium dodecyl sulphate extraction (Ikegami and Francki, 1975). Purified nucleic acid had ultraviolet spectra with 260/230 nm and 260/280 nm ratios of about 2.5 and 2.0, respectively, and gave positive diphenylamine reactions indicating the presence of deoxyribose (Shatkin, 1969). Single- and double-stranded DNAs differ in their behavior when heated or treated with formaldehyde (Miura *et al.*, 1966; Robinson and Hetrick, 1969; Sinsheimer, 1959). Upon treatment with formaldehyde (1.85, v/v) at room temperature, MYMV DNA exhibited the behavior expected of single-stranded (ss) molecule. A 5 nm shift in wave length maximum and approximately 18% hyperchromicity were observed within 10 min after addition of formaldehyde to purified MYMV DNA, whereas calf thymus DNA, which is double-stranded, did not show such a shift in wave length maximum and hyperchromicity. The ultraviolet light absorption of MYMV DNA increased over a broad range of temperatures from 20 to 70°C, whereas calf thymus DNA showed a sharp transition with a Tm of 77°C. Nucleic acid preparations from MYMV analysed by polyacrylamide gel electrophoresis in 7 M urea exhibited two bands (Fig. 4A). Nucleic acid of MYMV was treated with either DNase I or

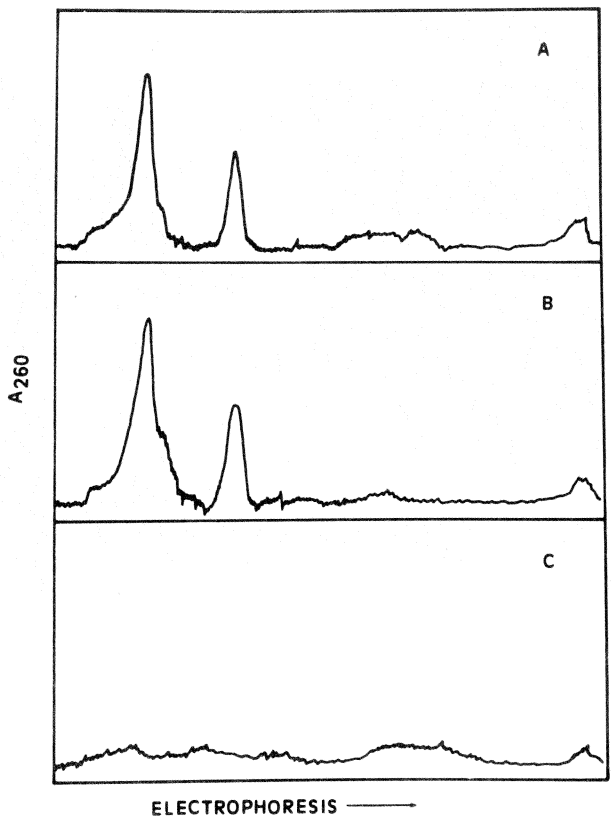

Fig. 4 Polyacrylamide gel electrophoresis of MYMV nucleic acid after nuclease treatments for 2 hr at 37°C. The samples were electrophresed by 4% polyacrylamide slab gel in 7 M urea at 80 V for 15 hr. (A) : Water; (B) : RNase A; (C) : DNase I.

bovine pancreatic RNase A, and analysed by polyacrylamide gel electrophoresis. Nucleic acid of MYMV was hydrolyzed by DNase I but not bovine pancreatic RNase (Fig. 4B, C). When the susceptibility of MYMV nucleic acid to nuclease S1 was tested, the digestion rate of MYMV nucleic acid was comparable to that of heat-denatured calf thymus DNA.

These results suggest that the nucleic acid of MYMV consists of ssDNA.

To examine the size of MYMV DNA, MYMV DNA preparation with ϕx174 DNA as the internal standard was examined in an electron microscope (Vollenweider *et al.*, 1975). The MYMV DNAs which diffused in pH 7.9 solution consisted predominantly of circular molecules and had a mean contour length of 0.93 ± 0.09 nm (n=150 molecules); circular ϕx174 DNA molecules which co-diffused in the same solution had a mean contour length of 1.97 ± 0.08 nm (n=50 molecules) (Fig.5). ϕx174 DNA consists of 5386 nucleotides (Sanger *et al.*, 1978) with a molecular weight of 1.72 (± 0.03) × 10^6 (Evenson, 1977). The corresponding value for MYMV circular DNA molecule was calculated as 2542 nucleotides with a molecular weight of 8.0 × 10^5.

All data presented here are consistent with the conclusion that MYMV comprises geminate particles and contains circular ssDNA. MYMV was thus assigned to the geminivirus group (Matthews, 1979).

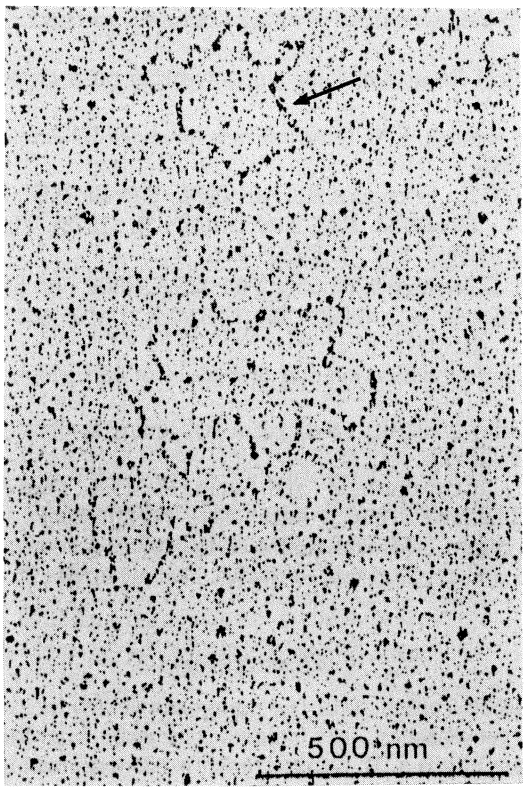

Fig. 5 Electron micrograph of MYMV nucleic acid (smaller molecule; arrow) and ϕx174 DNA (larger molecule). Both nucleic acids were mounted on a grid by the diffusion method.

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Discussion

Reddy, D.V.R. (ICRISAT): I believe that the mungbean yellow mosaic virus from India is different from that you observed in Thailand. The virus from India has been purified and found to be a geminivirus. It is not transmitted mechanically but is transmitted by whitefly and infects peanut.

Answer: There are differences between the Thai and Indian isolates of MYMV. The former is transmissible by mechanical inoculation unlike the latter. The host range of the Indian isolate is wider than that of the Thai isolate and includes Gramineae and Compositae in addition to Leguminosae. I believe that we are dealing with different strains of the same virus.