

Bean golden mosaic virus感染葉から分離したウイルスに特異的な2本鎖DNAの感染性

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Infectivity of Virus-Specific Double-stranded DNA from Tissue Infected by Bean Golden Mosaic Virus, a Geminivirus*

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Key words : bean golden mosaic virus, replicative form, double-stranded DNA, geminivirus.

Bean golden mosaic virus (BGMV) is one of a recently recognized group of plant viruses called geminiviruses which contain single-stranded (ss) DNA¹⁾. BGMV virions are geminate particles measuring about 18×30 nm, and each particle contains a single molecule of circular ssDNA of about 2.5Kb²⁾. Haber *et al.*³⁾ and Ikegami *et al.*⁴⁾ found that the sequence complexity of the BGMV genome, as revealed through restriction digest analysis, was about twice the size of a single-stranded DNA circle. They concluded that the BGMV genome must be divided, composed of two DNA circles of about 2.5Kb. The two component nature of the geminivirus genomes has been borne out by recent restriction maps produced for the BGMV⁵⁾ and tomato golden mosaic virus (TGMV) genomes⁶⁾ and by the DNA sequence analysis of the cassava latent virus (CLV) genome⁷⁾.

A BGMV-specific double-stranded (ds) DNA has been identified in BGMV-infected tissue and shown to contain the sequences of both types of genomic DNA. On the basis of denaturation and nuclease tests, this dsDNA was shown to have a circular structure with a discontinuity in one strand⁴⁾.

In this paper we present evidence which indicates that BGMV specific dsDNA infects bean plants. This is the first report of infectivity of BGMV dsDNA which is isolated from BGMV infected leaves.

BGMV was maintained in and purified from beans (*Phaseolus vulgaris* L. "Top Crop"). Freshly virus-inoculated plants were placed in a KOITO growth chamber operating at 30 C providing a 14-hr day length. Purification of BGMV was carried out as described by Goodman⁸⁾. BGMV ssDNA was isolated from purified BGMV particles by

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phenol-sodium dodecyl sulphate (SDS) extraction⁹⁾. BGMV ssDNA labeled with ^{32}P *in vivo* was obtained by collecting systemically infected trifoliolate leaves from plants 5 days after inoculation of primary leaves and feeding with ^{32}P -orthophosphate (100 μCi per leaf) through the cut ends of petioles. The leaves were then maintained for 4 days in a Petri dish by feeding a balanced inorganic salt solution through the petioles.

BGMV-specific dsDNA was isolated as follows⁴⁾. BGMV-infected leaves were frozen and ground to fine powder in liquid nitrogen. Cells were lysed at 69 C for 15 min by the addition of 200 mM Tris-HCl (pH 9.0), 200 mM EDTA, 4% SDS. Proteins were extracted with a 1:1 (v/v) mixture of phenol and chloroform. The aqueous phase was washed three times with phenol and chloroform. Nucleic acids were then precipitated from the aqueous phase with ethanol and resuspended in a buffer consisting of 50 mM Tris-HCl (pH 7.9), 50 mM NaCl, 5 mM EDTA. The contaminating proteins were removed by treatment with proteinase K at 1 mg/ml in 0.5% SDS for 15 min at 37 C. Proteins were extracted with phenol and chloroform twice and nucleic acids were precipitated with ethanol and solubilized in 0.12 M sodium phosphate (pH 6.8). DsDNA was purified from the total nucleic acids by hydroxyapatite (HA) column chromatography. The HA (Bio-Gel HPT, BIO-RAD) was equilibrated in a column (1.5 $\times$ 3 cm) with 0.12 M sodium phosphate (pH 6.8). The nucleic acid preparation was loaded and the column was washed with 0.12 M sodium phosphate (pH 6.8) until no absorbance at 260 nm and 280 nm was detected in the eluent. SsRNA and ssDNA were not absorbed on the HA column equilibrated with 0.12 M sodium phosphate (pH 6.8). The dsDNA preparation was layered on a 12 ml linear 5-20% (w/v) sucrose gradient in 10 mM Tris-HCl (pH 7.0), 10 mM NaCl, 1 mM EDTA, 0.1% SDS and centrifuged at 25,000 rpm for 17 hr in a Beckman SW 41 rotor. The virus-specific dsDNA in the middle (fraction 10-15 out of 26 fractions) of the gradient was collected and used for infectivity test.

Nuclease treatments of nucleic acid were carried out as follows unless otherwise provided. Nucleic acid (5 μg in 0.4 ml) was incubated with DNase I (5 μg), bovine pancreatic RNase A (2 μg), or nuclease S1 (2 units). Treatments were for 2 hr at 37 C in 10 mM sodium acetate, 5 mM magnesium sulfate (pH 5.0) (nuclease S1 treatments also included 0.5 mM ZnCl_2).

Polyacrylamide gel electrophoresis was carried out at room temperature in 0.6 $\times$ 5.6 cm cylindrical, 2.5% polyacrylamide gels containing 0.13% bisacrylamide, 20 mM Tris-HCl (pH 7.8), 2 mM EDTA. Voltage was applied providing 3 mA per gel for 6 hr.

The infectivity of BGMV specific dsDNA, purified as described above, was tested by rubbing the nucleic acid solution on expanding primary leaves of Top Crop leaves with celite powder. Ten to twelve days after this inoculation, a typical yellow mosaic symptom appeared (Table 1). Nuclease S1-treated BGMV dsDNA was also infectious. When plants were either mock-inoculated or inoculated with DNase-treated BGMV dsDNA, typical symptoms did not appear. The total nucleic acid was isolated from Top Crop leaves which showed the typical yellow mosaic symptom caused by BGMV dsDNA. Such

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nucleic acid was hybridized with ^{32}P -labeled BGMV ssDNA (Table 2). This result shows that BGMV dsDNA is present in BGMV dsDNA inoculated leaves

We examined whether or not virus particles could be detected in plants infected with BGMV dsDNA. Leaves harvested from BGMV dsDNA inoculated plants 6–12 days after inoculation were homogenized at 4 C in 0.1 M sodium phosphate (pH 7.8), 10 mM EDTA, 1 mM cysteine. The homogenate was strained, clarified by centrifugation, adjusted to 0.2 M sodium chloride, and 6 g of polyethylene glycol 6,000 was added per 100 ml clari-

Table 1. Infectivity of BGMV dsDNA

DNA source	Amount (μg)	Treatment	Plants infected/inoculated	
			1	2
BGMV ssDNA	10	None	10/10	10/10
	5	None	6/10	—
	10	DNase (100 μg) added and incubated at 37C for 2 hr	0/10	0/10
	10	Nuclease S1 (2units) added and incubated at 37C for 2 hr	0/10	0/10
BGMV dsDNA	10	None	6/10	7/10
	5	None	3/10	2/10
	0.1	None	1/10	1/10
	10	DNase (100 μg) added and incubated at 37C for 2 hr	0/10	0/10
	10	Nuclease S1 (2units) added and incubated at 37C for 2 hr	6/10	6/10

Table 2. Hybridization analysis of nucleic acid from BGMV dsDNA inoculated leaves with ^{32}P -labeled BGMV ssDNA^{a)}

Reaction condition	Hybrid yield (%)	
	Exp. 1	Exp. 2
Reanneal+ ^{32}P -BGMV ssDNA (780cpm), nuclease S1	95	93
Reanneal+ ^{32}P -BGMV ssDNA (1516cpm), nuclease S1	96	97
No melt+ ^{32}P -BGMV ssDNA (1516cpm), nuclease S1	0	0

- a) The nucleic acid from BGMV dsDNA inoculated leaves was melted by immersing for 3 min in boiling water and then quickly cooling in ice-water. The melted DNA was reannealed at 67 C for 17 hr with ^{32}P -labeled BGMV ssDNA (reaction mixture volume of 40 μl). Samples were divided into aliquots and one portion was incubated with 0.1 units of nuclease S1 at 37 C for 2 hr. Treatment of nuclease S1 was at pH 5.0 in sodium acetate, 5 mM magnesium sulfate, 0.5 mM ZnCl_2 . Enzyme-resistant DNA was precipitated with trichloroacetic acid, collected on filters, and assayed for radioactivity. The percentage of hybrid formation was calculated from a comparison of data from duplicate samples incubated either in the presence or absence of nuclease S1.

fied extract. The polyethylene glycol precipitate was collected by centrifugation after stirring for 2 hr at 4 C, and the pellet dissolved in a small volume of 0.1 M sodium phosphate (pH 7.8). The solution was subjected to two cycles of differential centrifugation ($17,500\times g$, 25 min followed by $257,000\times g$, 1.25 hr) and then to rate-zonal centrifugation on linear sucrose density gradients (36,000 rpm, 2.5 hr, Beckman SW 40 Ti rotor) (Fig. 1). A peak with position identical to native BGMV particles was observed. This peak can be observed in the tube containing geminate particles identical in morphology to native BGMV by electron microscopy (data not shown). Nucleic acid was extracted from virus particles which were purified from plants inoculated with BGMV dsDNA and analysed by electrophoresis in polyacrylamide gels (Fig. 2). Bands with mobilities identical to the circular and linear ssDNA components of BGMV were obtained. These bands were resistant to RNase A but could not be detected after digestion of the nucleic acid with DNase I or SI nuclease prior to electrophoresis confirming that they were ssDNA (data not shown). The linear band is believed to be derived from nicking of the circular component³⁾.

From all the data presented here, it is clear that BGMV dsDNA is infective and gives rise to progeny virus which is indistinguishable from native BGMV.

This observation suggests that whether or not BGMV ssDNA is first converted to

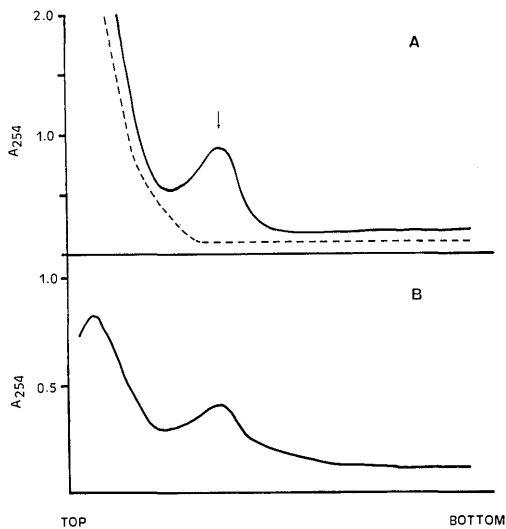


Fig. 1. Sucrose density gradient profiles of material isolated by differential centrifugation from (A) BGMV infected leaves (—) and healthy leaves (.....), and (B) BGMV dsDNA inoculated leaves. The sedimentation profiles shown were obtained by scanning the sucrose density gradient from the top with an ISCO density gradient fractionator coupled to a Model UA-5 ultra-violet analyser. An arrow indicates peak of BGMV.

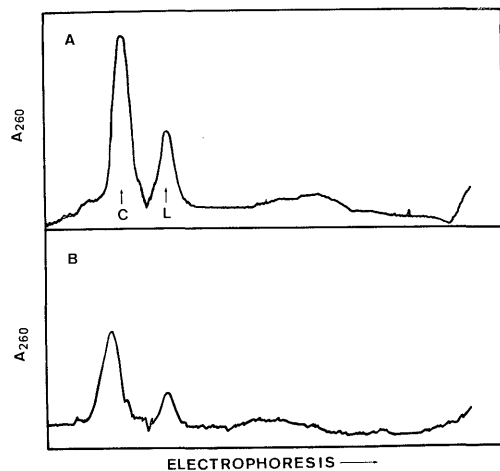


Fig. 2. Polyacrylamide gel electrophoresis of (A) nucleic acid of native BGMV particles and (B) nucleic acid of virus particles which were isolated from BGMV dsDNA inoculated leaves. The samples were electrophoresed in 2.5% polyacrylamide gel. Arrows indicate positions of circular (C) and linear (L) ssDNAs purified from BGMV particles.

ds form by cellular DNA polymerase, the RF isolated from infected cells is a component both in the transcription of viral mRNA and in DNA replication. At this early stage of knowledge of BGMV, this RF may be useful as a vehicle for introducing new genetic functions into higher plant cells although the possibility also exists for their use as vectors for cloning DNA in plant cells.

和 文 摘 要

池上正人・森永 傳・三浦謹一郎: bean golden mosaic virus 感染葉から分離したウイルスに特異的な2本鎖 DNA の感染性

bean golden mosaic virus (BGMV) 感染葉から、フェノール・SDS 法, ヒドロキシアパタイトカラムクロマトグラフィーおよびショ糖密度勾配遠心法によって, BGMV に特異的な2本鎖(ds) DNA を精製し, その感染性について調べた。0.1 μ g~10 μ g の BGMV dsDNA をインゲンに接種したところ, 接種後約2週間目に黄斑モザイク症状が観察された。このような罹病葉内には, BGMV ゲノム DNA と雑種形成する分子および BGMV 粒子が検出された。以上の結果から, インゲンに接種した BGMV dsDNA は接種葉内で複製を開始し, ウイルス粒子を形成するものと思われる。

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