Properties of a Strain of Bean Pod Mottle Virus

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ABSTRACT

J-10, a strain of bean pod mottle virus (BPMV), was efficiently transmitted by the bean leaf beetle, had the same host range as BPMV, and contained the three typical centrifugal components. J-10 differed from BPMV serologically and in the symptoms produced in *Chenopodium quinoa*. Separation of middle (M) and bottom (B) centrifugal components of both virus isolates and remixing increased lesion counts, whether the mixtures were homologous or heterologous. Soybean plants were inoculated with extracts from local lesions obtained from homologous and heterologous combinations of the M and B of J-10 and BPMV. The mixtures containing J-10 M produced infections which gave serological reactions characteristic of J-10 virus, indicating that the genetic information for the antigenic characteristics of the protein is carried by M. Phytopathology 61:831-833.

Additional key words: serology, component interactions.

Members of the squash mosaic virus family are beetle-transmitted, serologically distantly related, and exhibit three centrifugal components (2). The most rapidly migrating components, middle (M) and bottom (B), of two members of this family, bean pod mottle virus (BPMV) and cowpea mosaic virus (CPMV), are more infectious when components (from the same virus) are mixed than separate (6, 7).

No closely related strains of BPMV have been described. However, serological and host range studies of a virus isolate designated J-10, which was collected from soybean near Augusta, Ark., indicated that J-10 was related to BPMV. This work compares certain properties of J-10 with those of BPMV, and describes the interactions of their centrifugal components.

MATERIALS AND METHODS.—The J-10 isolate was passed through three single local lesion isolations on Pinto bean. The host range tested consisted of bean (*Phaseolus vulgaris* L. 'Pinto', 'Great Northern', 'Bountiful', 'Cherokee Wax', and 'Black Valentine'); cowpea (*Vigna sinensis* [Torner] Savi 'Monarch' and 'Early Ramshorn'); soybean (*Glycine max* [L.] Merr. 'Lee', 'Hill,' and 'Hood'); cabbage (*Brassica oleracea* L. var. *capitata* 'Early Jersey Wakefield'); tobacco (*Nicotiana tabacum* 'F_2C_1'); cucumber (*Cucumis sativus* L. 'Model'); and *Chenopodium quinoa*. Host range studies were performed under greenhouse conditions. Inoculations were made with sap extracted from infected soybean to plants dusted with Carborundum. Test plants which did not show symptoms 2 weeks after inoculation were back-inoculated to Pinto bean.

J-10 and BPMV were purified either from infected Lee soybean or Black Valentine or Cherokee Wax bean 10-14 days after inoculation. Infected plant tissue was harvested and frozen for 1 to 10 days. The frozen tissue was homogenized in a Waring Blendor with 0.2 M phosphate buffer, pH 7.2 (2 ml/g tissue), and an equal volume of a 1:1 mixture of chloroform and *n*-butanol. The emulsion was broken by low-speed centrifugation, and the supernatant was subjected to three cycles of high-speed centrifugation (80,000-100,000 *g* for 90 min). After each high-speed centrifugation, the pellets were resuspended in 0.1 M phosphate buffer, pH 7.2, and clarified by low-speed centrifugations at 8,000 *g* for 15 min.

J-10 and BPMV preparations were routinely examined in a Beckman Model E analytical ultracentrifuge. Sedimentation coefficients were estimated by Markham's graphical method (5).

Separation of virus components was achieved by layering virus preparations at a concentration of 5-6 A at 260 nm, 1 ml/tube, on linear gradients ranging from 0.2 to 1 M sucrose, made up in 0.01 M phosphate buffer, pH 7.2, and centrifuging in a Beckman SW-27 rotor for 3 hr at 81,000 *g*. The gradients were fractionated in an ISCO fractionator. The leading half of M and the trailing half of B were collected and pelleted by high-speed centrifugation and recycled once or twice on similar gradients. The components were adjusted to 0.025 A at 260 nm. Separated M and B and homologous and heterologous mixtures of M and B (equal volumes) were compared on half-leaves of Pinto bean. In order to determine which component or components carried genetic information for the antigenic characteristics, Lee soybeans were inoculated with single local lesions removed from Pinto bean infected with mixtures of components. Extracts from these plants were checked serologically 2 weeks after inoculation.

Gel-diffusion tests were performed in 1% agarose made up in 0.01 M phosphate buffer, pH 7.2, with 0.02% sodium azide. Bean pod mottle virus, squash mosaic virus (SMV), and CPMV antisera were used.

Bean leaf beetles, *Cerotoma trifurcata* (Forst.), were collected from soybean fields which appeared to be free from BPMV. The beetles were fed for 24 hr on healthy plants, placed on J-10 or BPMV-infected Lee soybean for a 24-hr acquisition feeding, then placed singly on caged healthy Lee soybeans for 24 hr. Plants were observed for 2 weeks for symptoms. Extracts from all plants were tested for virus on Pinto bean and in gel-diffusion plates.

RESULTS.—No differences in symptoms were observed between J-10 and BPMV in Pinto, Great Northern, Bountiful, and Cherokee Wax beans, in the

cowpea varieties, and in Lee and Hood soybean. However, Black Valentine bean and Hill soybean showed more severe symptoms when infected with J-10. Bean pod mottle virus caused obscure chlorotic lesions in *C. quinoa* and remained localized in the inoculated leaves, whereas J-10 caused chlorotic ringspots on inoculated leaves followed by systemic invasion characterized by yellow mottling. Cabbage, tobacco, and cucumber were not susceptible to either virus.

J-10 exhibited the typical top, middle, and bottom components of BPMV (Fig. 1). Sedimentation coefficients were 53, 88, and 109 S, which are quite similar to those determined for BPMV (1). Yields of purified J-10 were consistently 2 to 3 times higher than the yields of purified BPMV, based on absorbancies at 260 nm.

Spurs were formed by BPMV with J-10 in Ouchterlony gel-diffusion comparisons against BPMV antiserum (Fig. 2). Repeated injections of rabbits with purified J-10 resulted in high titer antisera (dilution end point greater than 1/2,048), but J-10 failed to form reciprocal spurs with BPMV in reactions against these antisera. When J-10 and BPMV in adjacent wells were diffused against SMV and CPMV antisera, the bands coalesced, but SMV and CPMV formed spurs with both isolates.

J-10 was readily transmitted by the bean leaf beetle. After feeding on the acquisition host, 32 beetles were placed individually on test plants. After 24 hr, 28 beetles had fed, and 8 transmitted the virus. In a parallel experiment with BPMV, 8 of the 24 beetles which fed transmitted.

Equal concentrations of J-10 and BPMV resulted in equal lesion counts; i.e., the specific activities of the two isolates are the same. Table 1 presents the results of one of several experiments to test the infectivity of M and B mixtures. Mixtures of J-10 M and B resulted in an increase in lesion numbers over lesion numbers obtained with either component alone. Bean pod mottle virus components behaved similarly. Heterologous mixtures (J-10 M + BPMV B, BPMV M + J-10 B) resulted in higher lesion counts than any component alone.

TABLE 1. Infectivities of separated and mixed middle and bottom components of the bean pod mottle virus and the J-10 strain

Component or mixture[a]	Lesion numbers[b]
J-10 M	1
M + B[c]	30
J-10 B	8
M + B	45
BPMV M	12
M + B	17
BPMV B	6
M + B	37
J-10 M + J-10 B	27
BPMV M + BPMV B	32
J-10 M + J-10 B	29
BPMV M + J-10 B	23
J-10 M + J-10 B	47
J-10 M + BPMV B	58
BPMV M + BPMV B	38
BPMV M + J-10 B	34
BPMV M + BPMV B	38
J-10 M + BPMV B	38

[a] Components were separated by density-gradient centrifugation and diluted to 0.025 A at 260 nm with 0.01 M phosphate buffer, pH 7.2, prior to inoculation to 8 Pinto bean half-leaves. Mixtures were composed of equal volumes of separated components.

[b] Average number of lesions per half-leaf.

[c] M and B = middle and bottom components, respectively.

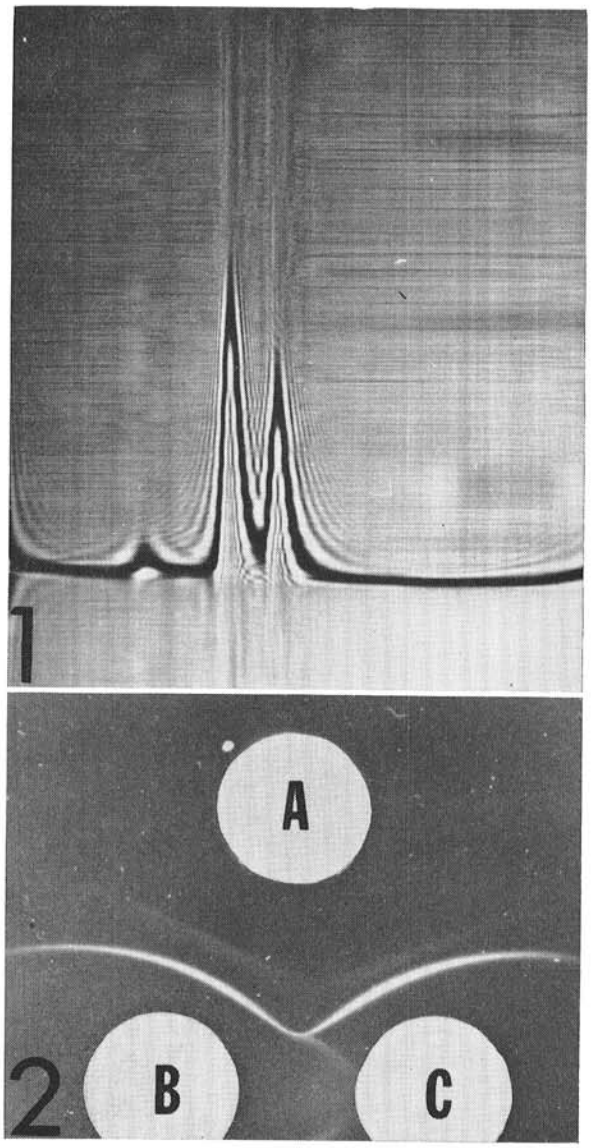

Fig. 1-2. **1)** Ultracentrifuge pattern of purified J-10 virus at a concentration of about 4 mg/ml suspended in 0.1 M phosphate buffer, pH 7.2. The photograph was taken 12 min after a speed of 32,000 rpm was reached in the Spinco Model E centrifuge. Schlieren angle = 60 degrees. **2)** Gel diffusion test showing the cross reaction of the J-10 strain with bean pod mottle virus antiserum; Well A contains bean pod mottle antiserum; Well B, bean pod mottle virus; Well C, J-10 strain.

In serological tests, isolates of J-10 M and BPMV B mixtures were placed in wells adjacent to BPMV, and reacted against BPMV antiserum. Spurs were evident in 36 of 49 cases. However, when isolates of BPMV M and J-10 B mixtures were placed in wells adjacent to BPMV and reacted against BPMV antiserum, the bands coalesced like isolates of BPMV M and BPMV B in 46 of 55 cases. We attribute inconsistencies in these data (such as 13 of 49 J-10 M + BPMV B mixtures which did not spur) to our inability to completely separate M and B components.

DISCUSSION.—Our data indicate that genetic information for BPMV capsid structures is carried by the middle component, as in most cases the serological difference between J-10 and BPMV can be brought about by addition of J-10 M to BPMV B and not by addition of J-10 B to BPMV M. Bruening (3) and De Jager and Van Kammen (4) demonstrated that middle component of CPMV was responsible for inheritance of top component production. Bruening postulated that top component is formed because of excess formation of capsid protein, and that middle component RNA carries the genetic information coding for polypeptide sequence of top, middle, and bottom components.

Based on increased infectivity in reciprocal mixtures of M and B components, J-10 is a closely related strain of BPMV. It is the first to be described. Van Kammen (6) reported that the phenomenon of component interaction was strain-specific, as mixtures of M and B from serologically related (but not identical) strains of CPMV resulted in no increase in lesion numbers. Wood & Bancroft (7) also reported that an increase in infectivity of BPMV occurred only when components of BPMV were mixed. Squash mosaic virus and components from CPMV did not increase the infectivity of either BPMV component. It is possible that "closeness" of relationship between multi-component viruses can be determined by the effect on infectivities of middle and bottom components of different strains when components of the strains are mixed.

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