Bean Pod Mottle Virus: Occurrence in Nebraska and Seed Transmission in Soybeans

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ABSTRACT

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A virus found infecting soybeans in Nebraska was shown to be seedborne in this host. Host range, in vitro properties, particle morphology, molecular weight estimates of the coat proteins, and sedimentation profile of the virus were typical of viruses in the comovirus group. Serological tests proved that the virus was bean pod mottle virus. This is the first record of this virus in Nebraska and the first report of seed transmission in soybeans.

Bean pod mottle virus (BPMV), a member of the comovirus group, causes a severe disease in soybeans (*Glycine max* (L.) Merr.) in the southern and eastern United States (14) and some midwestern states (4,9,11). No evidence has been obtained for seed transmission of this virus in soybean (12,13,15).

In August 1981, we received soybean plants showing severe leaf mottling from a seed company in east central Nebraska (Douglas County) for diagnosis. BPMV was identified in this sample, and the virus was shown to be seedborne in soybean. We report the characterization and seed transmission of this virus isolate.

MATERIALS AND METHODS

Soybean plants of two private varieties showing distinctive mottling of leaves were collected in east central Nebraska in early August 1981. Preliminary diagnostic procedures included electron microscopic examination of leaf-dip preparations in potassium phosphotungstate, pH 6.8, immunodiffusion tests against antisera to legume viruses, and mechanical inoculation to indicator plants, as described previously (7). The virus was freed from possible contaminating viruses by single-lesion transfers in bean (*Phaseolus vulgaris* L. 'Top Crop'). Host reactions, in vitro properties, and seed transmission were determined as reported previously (8). Soybean seeds used in the transmission study were from three different sources: 1) 1,200 seeds from the seed lot used to plant one of the fields where the virus was found, 2) 2,336 and 2,370 seeds of two private cultivars harvested from infected plants in the field, and 3) 2,270 seeds collected from 23 greenhouse-grown soybean cultivars previously inoculated with the virus. Seeds were planted in steam-sterilized soil in a greenhouse and fumigated at weekly intervals. No other legumes infected with BPMV were present in adjacent greenhouse rooms. Indexing procedures used bean cultivars Bountiful and Royalty Purple Pod as indicator plants, and agar gel immunodiffusion tests were performed to detect potential latent infection.

The virus was purified from soybean plants mechanically inoculated with the virus by the method of Moore and Scott (10) with the following modifications: 1) 8% *n*-butanol instead of a 1:1 mixture of chloroform and *n*-butanol was used for clarification, and 2) virus was initially precipitated with 6% polyethylene glycol (mol wt 6,000) instead of high-speed centrifugation. Concentration of the unfractionated virus preparation was determined spectrophotometrically by using $E_{260\ nm}^{0.1\%} = 8.7$ (14). Comparison of sedimentation patterns with serotypes I and II of cowpea severe mosaic virus (CPSMV) (6) and separation of purified virus components was by density-gradient centrifugation in a Beckman SW 27 rotor at 27,000 rpm for 3.5 hr. Gradients were prepared by layering 8 ml of 10, 20, 30, and 40% sucrose in 0.01 M phosphate buffer, pH 7.6, in centrifuge tubes (2.5 × 8.9 cm) and stored at 4 C overnight. Gradients were fractionated with an ISCO fractionator. Separation of the middle and the bottom components of the virus was according to Moore and Scott (10). Molecular weights of virus proteins were estimated in 10% polyacrylamide gels containing sodium dodecyl sulfate (5). Antiserum production and immunodiffusion in agar gel plates were as reported for CPSMV (6).

RESULTS

Initial identification and isolation of virus. Isometric particles about 26–30 nm were observed in leaf dips prepared from the soybean plants with mottled leaves (Fig. 1). When infected sap was tested in agar gel plates against the antisera to legume comoviruses kindly supplied by Fulton and Scott (3), a sharp, curved precipitin band occurred with BPMV antiserum, and a faint diffuse line was observed with antisera to CPSMV-Arkansas, bean rugose mosaic virus (BRMV), and quailpea mosaic virus (QPMV), but no reaction occurred with cowpea mosaic virus strain Sb (CPMV-Sb) antiserum.

Plants of the soybean cultivar Williams inoculated with sap from infected plants showed obscure chlorotic local lesions and systemic veinclearing in 4–5 days. The local lesions later became necrotic, and systemically infected leaves became severely mottled as observed in the field sample (Fig. 1). Serological tests confirmed the presence of BPMV in these plants. A virus isolate obtained after two single-lesion transfers in the cultivar Top Crop was used to characterize the virus designated as the Nebraska isolate.

Host reactions and in vitro properties. The Nebraska isolate, as well as the type and J-10 isolates of BPMV received from J. P. Fulton, University of Arkansas, infected 23 soybean cultivars or breeding lines (Beeson, Century, Calland, Coles, Cumberland, Davis, Hodgsen, Oakland, Pella, Wayne, Williams, Woodworth, A 1492, A 1564, A 2575, A 2656, A 3127, B 216, Amsoy, Corsoy, Vickery, Weber, and Wells) used in the Midwest and kindly supplied by A. D. Knapp, Iowa State University. The symptoms for all isolates were generally the same as described previously for the cultivar Williams, except that the type isolate induced only systemic symptoms. Amsoy, Corsoy, Vickery, Weber, and Wells appeared to be more tolerant than the others to all three virus isolates because they produced very mild symptoms that usually became masked after about 1 mo.

All three isolates caused chlorotic local lesions in Royalty Purple Pod, necrotic local lesions in the bean cultivar Blue Lake, and necrotic local lesions and systemic chlorotic spots in cowpea (*Vigna*

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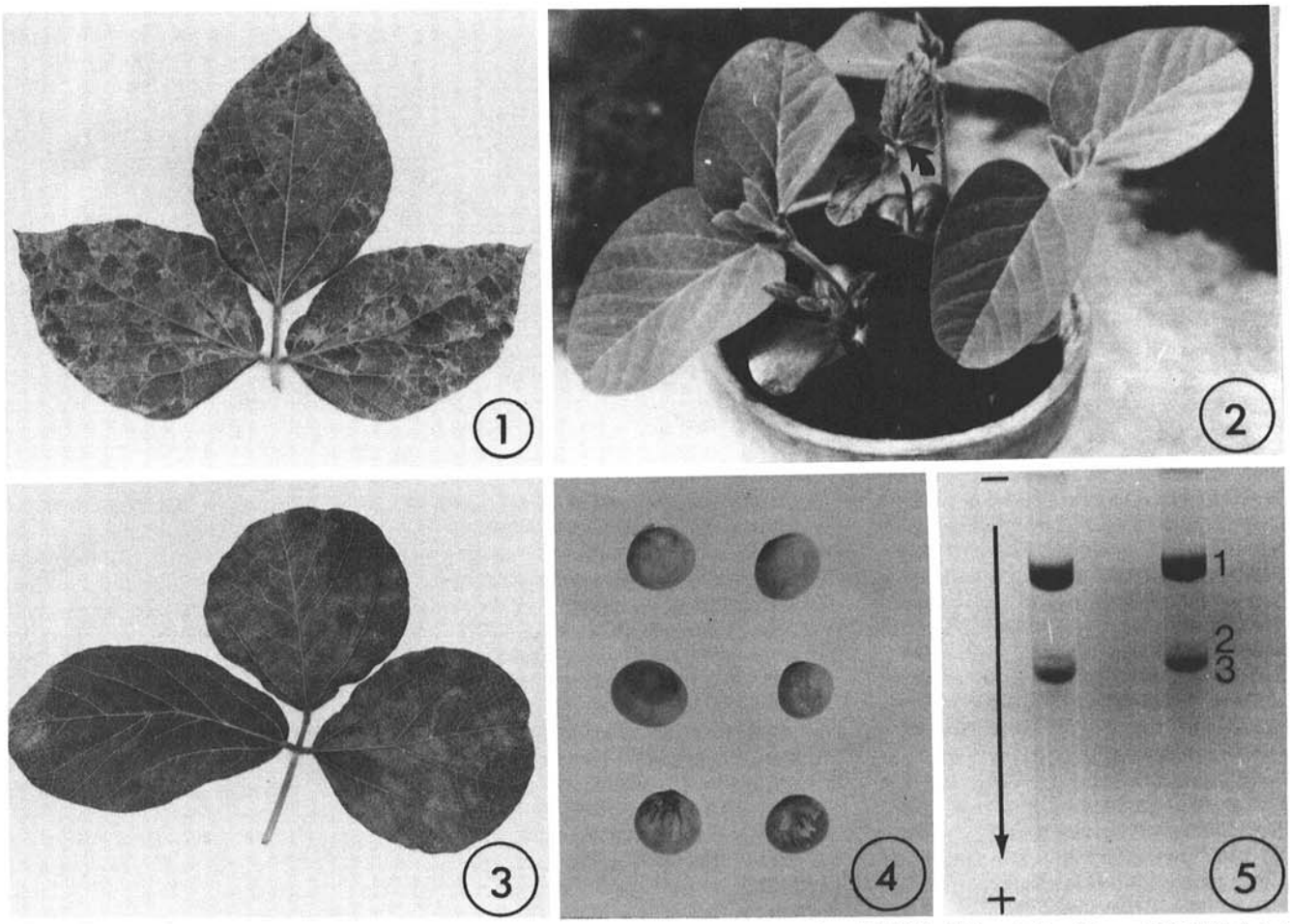
unguiculata (L.) Walp. subsp. *unguiculata* 'Blackeye Pea'). The Nebraska and type isolates, but not the J-10 isolate, induced chlorotic local lesions in bean cultivars Cherokee Golden Wax and Improved Tender Green, whereas the type, but not the other two isolates, caused systemic chlorotic spots in the bean cultivar Rico Pardo and obscure chlorotic local lesions in *Chenopodium quinoa* Willd. The following plants were immune to all three BPMV isolates: mung bean (*Phaseolus aureaus* Roxb.), yard long bean (*Vigna unguiculata* (L.) Walp. subsp. *sesquipedalis* (L.) Verdc.), lima bean (*Phaseolus lunatus* L. 'Henderson Baby Bush', *Gomphrena globosa* L., pea (*Pisum sativum* L.) 'Green Arrow' and 'Wondo', and the bean cultivar Slenderette. The major differences in host reactions among the three isolates are summarized in Table 1. The Nebraska isolate also induced veinal necrosis and necrotic local lesions in the bean cultivars Michelite, Great Northern, Red Mexican U134, Bountiful, and Top Crop but did not infect *Cassia obtusifolia* L., cowpea cultivars and breeding lines Serido, Macaibo, TVu 612, TVu 1948, TVu 2480, and PI 186465.

The Nebraska isolate had a dilution end point between 10^{-4} and 10^{-5} and a thermal inactivation point between 70 and 75 C.

Seed transmission. A total of 8,176 seeds were planted from three sources. Two and four seedlings from the two cultivars of source B developed BPMV symptoms of mild veinclearing and chlorotic spots in the primary leaves about 10 days after planting. Symptoms developed into severe mosaic, and the seedlings became stunted (Fig. 2). The newly formed trifoliate showed mild mosaic (Fig. 3). Sap of these seedlings did not react with antisera to soybean mosaic and tobacco ringspot viruses but formed a sharp band with the BPMV antisera in agar gel plates. No serological differences were observed between the seedborne virus isolates and the Nebraska isolate when tested against antiserum to the Nebraska isolate. Bioassay in Royalty Purple Pod bean and Williams soybean also confirmed the presence of BPMV in the seedlings. The seedborne isolates gave chlorotic and necrotic local lesions in bean cultivars Cherokee Golden Wax and Improved Tender Green, characteristic of the Nebraska isolate (Table 1). The 2,270 seeds from source C exhibited seed-coat mottling similar to field-grown seeds of source B (Fig. 4). BPMV was detected in one of the seedlings (cv. Williams), grown from these seeds.

Virus purification and gel electrophoresis. A purified, unfractionated virus preparation had an ultraviolet spectrum typical of nucleoprotein, with maximum and minimum absorptions at 258 and 239 nm, respectively. The A 260/280 was 1.78, and A max./min. was 1.50. Yield of the virus was 145 mg/kg of leaf tissue. Sucrose density-gradient sedimentation patterns revealed three bands located at about the same depth as the top (T), middle (M), and bottom (B) components of the CPSMV serotypes I and II. In contrast with the CPSMV preparations, which had a relatively high amount of M component, the Nebraska BPMV isolate had a large quantity of B component and very little T component. No light-absorbing material was detected near the meniscus of the gradient, indicating that the preparation evidently was free of contamination.

In gel electrophoresis experiments, the M and B components showed identical protein patterns with two major bands (nos. 1 and 3) and one minor (no. 2) band (Fig. 5). The minor band was resolved as a shoulder in photometric scanning



Figs. 1–5. (1) Mottled leaves of soybean naturally infected with the Nebraska bean pod mottle virus (BPMV). **(2)** Pot with healthy and BPMV-infected (arrows) soybean seedlings in seed transmission trial. **(3)** Newly formed leaves of seed-transmitted BPMV-infected seedling. **(4)** Mottled seeds collected from BPMV-infected soybeans. **(5)** Protein profiles of the middle (right) and bottom (left) components of the Nebraska BPMV in 10% polyacrylamide gels containing sodium dodecyl sulfate. Arrow indicates the direction of protein migration during electrophoresis.

profiles of the gels (Fig. 6). The apparent molecular weights of the three proteins in M component were estimated to be 38,683 ± 231, 22,304 ± 203, and 19,951 ± 222 (mean and standard deviation of five determinations), respectively, for the nos. 1, 2, and 3 proteins. The molecular-weight estimates for the proteins in the B component were 39,363 ± 255 (four determinations), 22,307 (one determination only), and 20,021 ± 112 (four determinations).

Serology. Like other comoviruses, the Nebraska isolate was highly immunogenic. An antiserum produced 1 mo after injecting a rabbit with 4 and 5 mg of a partially purified virus preparation at a 6-day interval, respectively, had a homologous titer of 1/256 in immunodiffusion tests. A 1:16 dilution of this antiserum, which formed a sharp band with its homologous antigen and did not react with the sap of healthy soybean, was used in the routine immunodiffusion tests. Compared with the type and the J-10 isolates, no serological differences were observed among the three isolates (Fig. 7).

DISCUSSION

The symptomatology, host range, and in vitro properties of the Nebraska isolate closely resembled those of BPMV (14). The isolate had a limited host range and infected only legumes. It induced mottled leaves and seeds in soybean, caused chlorotic or necrotic local lesions in most varieties of bean tested, and had the same thermal inactivation and dilution end points as reported previously for BPMV. It differed slightly, however, from the type and the J-10 isolates in some host reactions (Table 1).

The Nebraska isolate had three centrifugal components typical of comoviruses (2). In gel electrophoresis experiments, two major and one minor protein bands were observed in the M and B components. Blevings and Stace-Smith (1) reported similar patterns with broad bean true mosaic virus and concluded that the protein coat of this virus is made up of two polypeptides of molecular weights 37,500 and 20,000–24,500. Changes in mobility of the smaller polypeptide were ascribed to limited proteolysis. If interpreted in a similar manner, the protein coat of the Nebraska isolate contains two polypeptides with molecular weights of about 39,000 and 20,000–22,000. These are within the range reported for comoviruses (2).

The Nebraska isolate cross-reacted with antisera to three viruses known to be closely related to BPMV (CPSMV, QPMV, and BRMV [2]) and was serologically identical to the type and the J-10 isolates. On the basis of these results, the virus from Nebraska was identified as an isolate of BPMV. Although the virus has been reported to occur naturally in three midwestern states including Iowa (11), Kansas (4), and Illinois (9), this is the first report of BPMV in Nebraska.

Our results also demonstrated that the Nebraska isolate was transmitted through soybean seeds. Although Schwenk and Nickell (13) showed that BPMV could be serologically detected in seeds from infected soybean plants, none of the 537 and 1,089 seeds grown from infected plants in two trials produced infected seedlings. Other workers (12,15) also failed to demonstrate the seedborne nature of BPMV in soybean. According to Stace-Smith (16), members of the comovirus group are characterized by a low level of seed transmission that is difficult to detect. The Nebraska isolate had a 0.10% seed transmission rate (seven of 6,976 seeds). Therefore, if fewer seeds were used in the trial, the seedborne nature of this virus might have been overlooked.

Perennial legumes and overwintering beetle vectors are regarded as the sources of BPMV for primary infection in commercial plantings (16). With the present finding, infected soybean seed also can serve as the source of primary inoculum in the field. The epidemiological significance of this discovery is unknown; however, because BPMV has not been found in countries outside the United States, precautions are necessary to avoid the introduction of this virus to other parts of the world through seeds.

Table 1. Differences in host reactions among three isolates of bean pod mottle virus

Plant	Bean pod mottle virus		
	Nebraska	Type	J-10
Soybean (23 cultivars)	$LL_{c,n}$/VC,M[a]	−/VC,M	$LL_{c,n}$/VC,M
Bean cultivars Cherokee Golden Wax and Improved Tender Green	$LL_{c,n}$/−	LL_c/−	−/−
Bean cultivar Rico Pardo	−/−	−/CS	−/−
Chenopodium quinoa	−/−	LL_c/−	−/−

[a] Numerator and denominator indicate local and systemic symptoms, respectively. Symbols for the symptoms are: − = no symptoms and no virus recovered, LL_c = chlorotic local lesions, $LL_{c,n}$ = chlorotic and necrotic local lesions, VC = veinclearing, M = mottling, and CS = chlorotic spots.

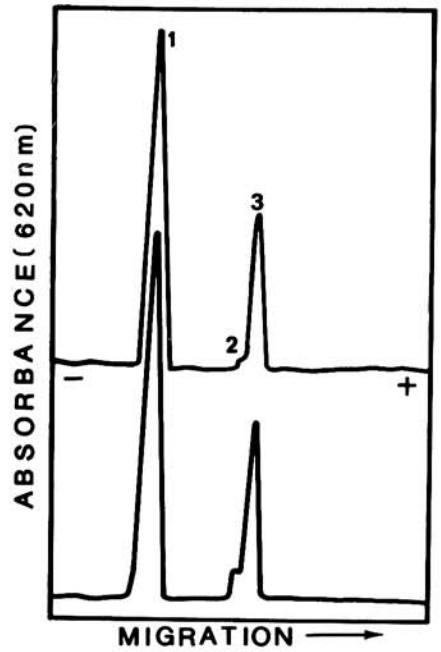


Fig. 6. Photometric scanning patterns of the polyacrylamide gels in Figure 5 showing the protein profiles of the middle (upper pattern) and bottom (lower pattern) components.

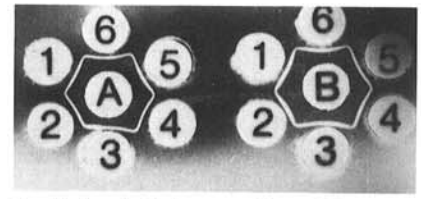


Fig. 7. Serological comparison of bean pod mottle virus (BPMV) isolates in agar gel plates. A and B = antisera to the Nebraska and J-10 isolates, respectively, at 1:16 dilution; 1 and 2 = sap containing the Nebraska isolate, 3 and 4 = sap containing the type isolate, 5 and 6 = sap containing the J-10 isolate. No spur formation was observed.

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