Bud Blight of Soybeans Caused by Cowpea Severe Mosaic Virus in Central Brazil

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ABSTRACT

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Plants of the soybean cultivar IAC-2 showing symptoms of bud blight in central Brazil were found to be infected by an isolate of cowpea severe mosaic virus (CPSMV) serotype I. The isolate was transmitted by the beetle *Cerotoma arcuata* but not through seeds of soybean IAC-2. Biological and in vitro properties of the Brazilian isolate were typical of CPSMV. Significant reductions in plant height, grain yield, number of pods per plant, and seed germination occurred in a field trial.

Bud blight symptoms were observed in plants of the soybean (*Glycine max* (L.) Merr.) cultivar IAC-2 at the Biological Station of the University of Brasilia (BSUB) in central Brazil in 1979. Bud blight of soybean has been reported to be caused by tobacco streak virus (TSV) in Brazil and by tobacco ringspot virus (TRSV) (13) or cowpea severe mosaic virus (CPSMV) (12,14) in the United States. Preliminary tests indicated that these plants at the BSUB were infected with an isolate of CPSMV.

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This paper describes the isolation, host reactions, in vitro properties, insect and seed transmission, purification, antiserum production, and serotyping of this virus isolate. The effects of this virus on the growth and yield of soybeans in the field are also reported. Preliminary results have been published (2).

MATERIALS AND METHODS

Soybean plants with bud blight symptoms were collected from two experimental plots at the BSUB in July of 1979. The plots were near a planting of cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) containing plants heavily infected with CPSMV and where the incidence of the beetle *Cerotoma arcuata* (Oliv.) was high. Sap extracted from infected soybean plants was divided into two parts. One part was tested in agar gel plates against antisera to TSV, three strains of TRSV (Iris, Cherry, and Blackberry), and the Arkansas isolate of CPSMV (CPSMV-Ark), supplied by R. W. Fulton (University of Wisconsin), R. Stace-Smith (Agriculture Canada), and J. P. Fulton (University of Arkansas), respectively. The other part was mechanically inoculated to a range of indicator plants including *Chenopodium quinoa* Willd., which developed necrotic local lesions within 3–4 days. A single lesion from *C. quinoa* was removed and inoculated to soybean cultivar IAC-2, which developed systemic symptoms. This single-lesion isolate maintained in IAC-2 soybean and designated as CPSMV-NB was used as the inoculum source in this study.

Mechanical inoculation, host range study, determination of in vitro properties, seed transmission, virus purification, antiserum production, serological tests, and serotyping were performed as reported earlier (10,11). Sedimentation coefficients of the virus components were estimated by sucrose density gradient centrifugation (4) using a tomato isolate of tobacco mosaic virus (TMV) as the standard for comparison. Purification of TMV was done by clarification with *n*-butanol, precipitation with polyethylene glycol (mol wt 6,000), and differential centrifugation. A value of 194 *s* was used for TMV (16).

For insect transmission trials, adults of *C. arcuata* collected from the cowpea field at the BSUB were used. The beetles were transferred seven times at 2-day

intervals to healthy soybean plants to free them from possible contamination of CPSMV in the field. The insects, in groups of five, were then allowed an acquisition feeding of 48 hr on plants of the cowpea cultivar Seridó infected with CPSMV-NB. Each group was used to consecutively inoculate seven groups of five healthy soybean IAC-2 plants with inoculation feeding periods of 48 hr. Eleven groups of beetles were used in two trials; 385 plants were inoculated. After 3 wk, plants were assayed for CPSMV by serology and inoculation in *C. quinoa*.

A field experiment to determine effects of this virus isolate on growth and yield of soybean IAC-2 was conducted at the BSUB (altitude of about 1,000 m), using a completely randomized design with 11 treatments and three replicates. Treatments were virus-inoculated and buffer-inoculated plants at each of five ages (14, 28, 42, 56, and 70 days after planting) and an uninoculated control. Each treatment consisted of 90 plants. Row spacing was 0.9 m. The experiment area was surrounded (at a distance of 1 m) by a border row of healthy soybean plants. Plant height (95 days after planting), grain yield, mean weight per 100 seeds, number of pods per plant, and percentage of seed germination were evaluated. Treatments, with the exception of seed germination, were statistically analyzed using Duncan's multiple range test ($P = 0.05$). Four hundred each of seeds collected from soybeans previously inoculated with the virus at 28, 42, 56 or 70 days after planting were tested for possible transmission of this isolate through seeds.

RESULTS

Field occurrence, isolation, in vitro properties, and host reactions. Eight of about 1,000 soybean plants in the experimental plots for physiological study at the BSUB showed bud blight symptoms. These plants were severely stunted compared with other apparently healthy plants. Crude sap of these plants formed a sharp, curved precipitin band with the CPSMV-Ark antiserum, but not with antisera to TSV and TRSV, in agar gel plates. This indicated infection by CPSMV. The CPSMV-NB isolate had a dilution end point of 10^{-4}–10^{-5} and a thermal inactivation point of 65–70 C. In crude sap, CPSMV-NB was infective for 9 (but not 10) days at room temperature.

In inoculation tests, all of the following 15 soybean cultivars and plant introduction (PI) lines became infected: IAC-2, Biloxi, Bossier, Bragg, Davis, UFV-1, IAS-4, Lincoln-1, Missão, Otootan, Paraná, Santa Rosa, PI 204339, PI 205913, and PI 320563. Initial symptoms in inoculated leaves were chlorotic or necrotic local lesions, followed by systemic mosaic and vein necrosis in trifoliolate leaves (Fig. 1A). Plants eventually became bud-blighted and severely stunted (Fig. 1B) as observed in the field. All 16 cowpea cultivars and breeding lines tested, ie, Branquinho, ER-1, Jaguaribe, IPEAN V-69, IPEAN VII, VITA-1, VITA-4, Rita Joana, Carrapicho, Alagoas, Sempre Verde, Rubi, Bengala, Pitiúba, Seridó, and TVu 4536, were susceptible to CPSMV-NB, producing chlorotic or necrotic lesions, stunting, and severe mosaic. Plants of soybean PI 320563 and cowpea cultivars VITA-1, VITA-4, ER-1, and Jaguaribe were highly susceptible to this isolate and were killed 12–14 days after inoculation. Bean (*Phaseolus vulgaris* L.) cultivar Manteiga reacted with chlorotic local lesions that became necrotic, severe systemic necrosis, and deformation of young leaves. In the Chenopodiaceae, CPSMV-NB induced pinpoint necrotic local lesions in *C. amaranticolor* Coste & Reyn., necrotic local lesions, systemic necrotic spots and severe leaf distortion in *C. quinoa* Willd. and *C. murale* L.

Purification and serology. CPSMV-NB was purified easily from leaves of cowpea Seridó, with a yield of 294 mg/kg. The absorption spectrum of the purified preparation had maximum and minimum absorptions at 260 and 240 nm, respectively, and $A_{260/280}$ of 1.6. In sucrose density gradients, the preparation separated into one faint and two prominent opalescent bands with estimated sedimentation coefficients of 58, 89, and 115 *s*, respectively.

An antiserum with a homologous titer of 1/1,024 in an immunodiffusion test was obtained from a rabbit 2 wk after the animal had been injected intramuscularly

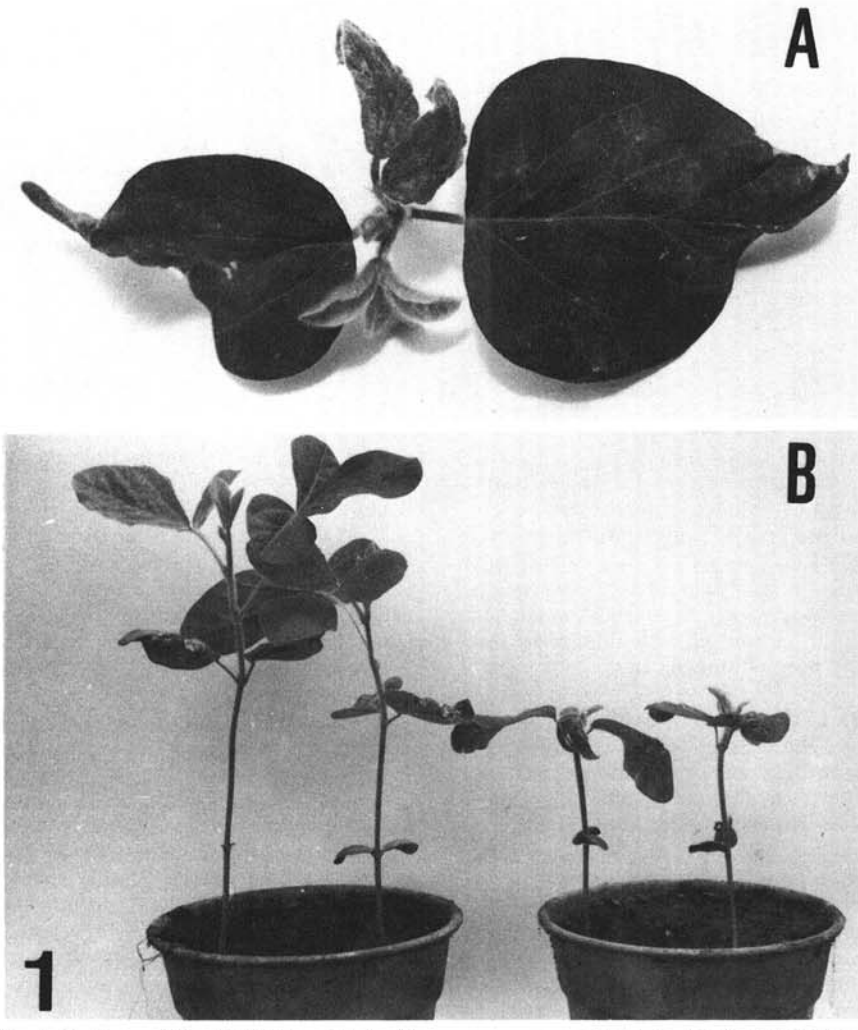

Fig. 1. Soybean cultivar IAC-2 inoculated with cowpea severe mosaic virus showing **(A)** chlorotic local lesions in inoculated leaves and systemic mosaic in young leaves and **(B)** bud blight and severely stunted plant (right) compared with a plant inoculated with buffer only (left).

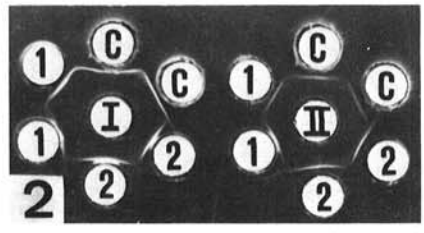

Fig. 2. Serotyping of the soybean isolate of cowpea severe mosaic virus (CPSMV) in agar gel plate. I and II = antisera to serotypes I and II, respectively; 1 and 2 = crude saps containing serotypes I and II, respectively; C = crude sap containing the soybean isolate of CPSMV.

Table 1. Effects of cowpea severe mosaic virus on growth and yield of soybean cultivar IAC-2 at different ages in the field[x]

Plant age (days) when inoculated	Inoculum[y]	Plant height (cm)	Grain yield (g/plant)	Grain weight (g/100 seeds)	Pods per plant (%)	Seed germination (%)
14	Buffer	86.30 a[z]	23.72 a	19.00 a	88.56 a	93.7
28	Virus	36.27 c	0.51 d	12.10 c	2.63 c	28.0
28	Buffer	85.65 a	23.23 a	18.10 b	92.30 a	92.0
42	Virus	38.97 c	1.41 d	12.10 c	12.16 c	29.2
42	Buffer	84.53 a	23.10 a	18.80 ab	91.83 a	95.0
56	Virus	64.55 b	5.05 c	12.90 c	40.33 b	32.2
56	Buffer	86.60 a	24.25 a	19.36 a	92.53 a	91.2
70	Virus	84.32 a	16.84 b	18.00 b	89.76 a	85.2
70	Buffer	84.45 a	24.09 a	19.33 a	90.00 a	96.8
...	Not inoculated	86.00 a	23.36 a	18.93 a	90.73 a	92.5

[x]Plants inoculated with virus when 14 days old died prematurely.
[y]Inoculum was crude sap containing the virus in 0.01 M phosphate buffer pH7.6 or buffer only.
[z]In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

with 11 mg of a purified CPSMV-NB preparation. The antiserum did not react with crude sap from healthy cowpea or soybean plants in agar gel plates. CPSMV-NB formed a sharp line that fused completely with that of serotype I, but spurred with serotype II, when tested against the serotype I antiserum. When serotype II antiserum was used, serotype II spurred with CPSMV-NB and serotype I (Fig. 2). The same results were obtained when serotype I antiserum was replaced with CPSMV-NB antiserum, indicating that CPSMV-NB is serologically identical to serotype I.

Insect and seed transmission. *C. arcuata* fed very well, making many holes in leaves of soybean IAC-2 plants. It transmitted CPSMV-NB from cowpea to soybean with a very low efficiency; among the 55 plants inoculated in each of the seven consecutive feedings, only one plant each in the first two feedings became infected. In the seed transmission trial, only 688 of the 1,600 seeds germinated (43% germination) and none was infected with CPSMV-NB.

Effects on growth and yield. In the field experiment, plants inoculated 14 days after planting were killed within 20–25 days. Plants inoculated at later stages of development survived to produce seeds, but their height, grain yield, grain weight, number of pods per plant, and seed germination rate were greatly reduced (Table 1). Reductions were greatest in plants inoculated at younger stages.

DISCUSSION

Although bud blight of soybean caused by CPSMV has been reported in the United States (12,14), this disease is generally considered to be caused by TSV in Brazil (13). This study reports the first incidence of this disease caused by CPSMV in Brazil. The host reactions, in vitro properties (with the exception of longevity in vitro), beetle transmission, and virus components of CPSMV-NB were in agreement with those described for CPSMV (8,10) and it was identified as a member of the serotype I of this virus, which is common in central Brazil (10).

Natural infection of soybeans by CPSMV has been reported in Trinidad (6), Puerto Rico (14), and Illinois (12) and now for the first time in Brazil. In mechanical inoculation tests, soybeans were susceptible to CPSMV (1,7,10,12) and soybean cultivar IAC-2 was previously suggested as a differential for serotypes I and II (10). In this study, all 15 soybean cultivars were susceptible to this isolate by mechanical inoculation. In spite of this, transmissions of this isolate from cowpea to soybean by *C. arcuata*, a known vector of CPSMV in Brazil (3,5), were few. Similar results were obtained by other authors (9,12). This has been suggested to be caused by the presence of inhibitors (9) or morphological and anatomical plant factors in soybean (12).

Field experiments conducted in Puerto Rico (15) showed that a CPSMV isolate could cause significant yield reductions in soybeans when 25% or more of the plants were inoculated at the primary leaf stage or when 50% or more of the plants were inoculated at midbloom stage. We also showed that the virus isolate severely damaged growth and yield of soybean IAC-2 (Table 1). Inoculation of 14-day-old soybean seedlings resulted in death of the plants within 20–25 days, and plants inoculated 28, 42, and 56 days after planting had significant reductions in plant height, grain yield, grain weight, number of pods per plant, and seed germination. Even plants that were inoculated 70 days after planting had a 30% reduction in grain yield. This loss apparently was due to reduction of the number of seeds per pod, because the grain weight and the number of pods per plant from these plants had very little reduction.

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