

Occurrence of a Strain of Bean Rugose Mosaic Virus on Soybean (*Glycine max*) in Central Brazil

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Abstract

A virus was isolated from a soybean plant showing mottling that was collected in a field of EMBRAPA/CPAC, DF, Brazil, in 1989. The plants showing systemic symptoms by mechanical inoculation of that virus were limited to the Leguminosae. *Chenopodium* spp. produced local lesions on the inoculated leaves. The virus was transmitted by beetles, *Cerotoma* sp. and *Diabrotica* sp. No seed transmission of the virus was observed in soybean and French bean. The thermal inactivation point, dilution end point and longevity in vitro of the virus were 60-65°C for 10 min, 10⁻⁴-10⁻⁵ and over 6 days at 20°C, respectively. The virus particles were isometric with about 30 nm in diameter, virus-like particles forming crystalline aggregates were observed under an electron microscope. The virus reacted positively with antiserum against bean "mosaico-em-desenho" virus, that is a strain of bean rugose mosaic virus. It also reacted weakly with antiserum against cowpea severe mosaic virus. Based on the criteria including host range, symptomatology, transmission mode, particle morphology and serological affinities observed, the virus was identified as a strain of bean rugose mosaic virus, belonging to comovirus.

Discipline: Plant disease

Additional key words: comovirus, host range, serology, transmission

Introduction

Soybean plants showing mottling with puffy areas among the veins of the leaves were observed and collected in 1989 in the field of Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA)/Centro de Pesquisa Agropecuaria dos Cerrados (CPAC) located in Planaltina, DF, Brazil. The virus had similar properties to those of bean "mosaico-em-desenho" virus isolated from French bean⁵⁾. Lin, et al. reported that the virus was identified serologically as a comovirus belonging to the serogroup of bean rugose mosaic virus (BRMV), and concluded that it was a strain of BRMV⁶⁾. The field surveys by the authors indicated that this virus disease was wide-

spread and it would probably be one of the most important virus diseases in French bean. It was also found that this virus infected soybean plants in Central Brazil. However, host range, transmission and other detailed properties of this virus have to be confirmed yet in Brazil.

The present paper attempts to describe some properties of the virus isolated from soybean in Central Brazil, with emphasis placed on the transmission by beetles and the serological relationship with bean "mosaico-em-desenho" virus which is a strain of BRMV.

Materials and methods

An original virus source was isolated from natu-

Table 1. Host range of a strain of BRMV

Plant species (cultivar)	Symptoms ^{a)}	
	Inoculated leaves	Uninoculated leaves
<i>Glycine max</i> (Cristalina)	-	M
<i>G. max</i> (Doko)	-	M
<i>Phaseolus vulgaris</i> (Carioca)	L	M
<i>P. vulgaris</i> (Bountiful)	L	M
<i>P. vulgaris</i> (Rico 23)	L	N
<i>P. lunatus</i> (Henderson Bush)	L	cs
<i>Pisum sativum</i> (Kinusaya)	-	M
<i>Vigna angularis</i> (Dainagon)	-	-
<i>V. radiata</i>	-	-
<i>V. unguiculata</i> (Blackeye)	-	-
<i>V. unguiculata</i> (IPEAN)	-	-
<i>Chenopodium amaranticolor</i>	L	-
<i>C. quinoa</i>	L	-
<i>Nicotiana benthamiana</i>	-	-
<i>N. clevelandii</i>	-	-
<i>N. glutinosa</i>	-	-
<i>N. tabacum</i> (Xanthi nc)	-	-
<i>Petunia hybrida</i>	-	-
<i>Physalis floridana</i>	-	-
<i>Gomphrena globosa</i>	-	-
<i>Sesamum indicum</i>	-	-
<i>Tetragonia expansa</i>	-	-
<i>Vinca rosea</i>	-	-
<i>Citrullus vulgaris</i>	-	-
<i>Cucumis melo</i>	-	-
<i>C. sativus</i>	-	-

a): L; Local lesion, M; Mosaic mottling, N; Necrosis, cs; Chlorotic spot, -; No symptoms.

rally infected soybean plants collected in the field of CPAC, Brazil, in 1989. The isolated virus was maintained in French bean and soybean plants by successive mechanical inoculations in a greenhouse. The inoculation was made by rubbing the carborundum (600 mesh)-dusted leaves with a piece of cotton dipped in the crude sap of infected leaves, which were homogenized in 0.1 M phosphate buffer (pH 7.2) containing 0.5% 2-mercaptoethanol.

Host range of the virus was determined by mechanical inoculations to various test plants (Table 1) in a greenhouse. Physical properties of the virus were determined by using crude sap from infected leaves of French bean prepared in 0.01 M phosphate buffer (pH 7.2).

In insect transmission tests, the beetles under testing, *Ceratoma* sp. and *Diabrotica* sp. were allowed on acquisition access for 2 days on infected French bean plants. These insects were mounted to healthy

French bean or soybean plants, and were allowed on inoculation access for feeding for one day. Ten beetles to 5 plants were used for each test.

For electron microscopy, partially purified preparation of the virus was negatively stained with uranyl acetate. The specimens were prepared from small pieces of the infected French bean leaves fixed in 3% glutaraldehyde and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2). Then, after dehydration through acetone series, the preparations were embedded in Spurr resin. Ultrathin sections were prepared by cutting with glass knives and stained by uranyl acetate and lead citrate. These specimens were observed under a Hitachi Model H-7000 electron microscope in Japan.

Serological tests with an agar gel double diffusion method were carried out by using 0.8% agar prepared in 0.05 M phosphate buffer (pH 7.2) containing 0.85% NaCl and 0.05% sodium azide.

Results

Host range and symptoms: The virus infected six plant species in two families among the tested 22 species in eight families (Table 1).

Glycine max showed mottling with puffy symptoms among the veins of the leaves in a field. In inoculation tests, soybean plants showed vein-clearing and mottling systemically. Subsequently these symptoms became indistinct, and sometimes chlorotic blotches appeared on the leaves. Twenty-nine varieties were inoculated with the above noted virus and all the varieties were infected systemically.

Phaseolus vulgaris showed faint chlorotic spots on the inoculated leaves. Systemically, vein-clearing and characteristic mosaic mottlings with irregular dark green islands along the veins appeared on the upper leaves. The mottlings with dark green spots developed on the pods. Eighteen varieties tested were all infected systemically.

P. lunatus showed faint chlorotic local lesions on the inoculated leaves, and chlorotic spots took place on the upper leaves. Later, these symptoms mostly disappeared.

Pisum sativum showed systemic vein-clearing and chlorotic mottling.

Chenopodium amaranticolor and *C. quinoa* showed chlorotic local lesions on the inoculated leaves without systemic infections.

Transmission: Two species of beetles, *Cerotoma* sp. and *Diabrotica* sp. transmitted the virus from diseased French bean to healthy bean plants, and the *Cerotoma* sp. transmitted the virus from diseased bean to healthy soybean plants (Table 2). The virus was not transmitted through soybean (cv. Doko) seeds (0/74) and French bean (cv. Carioca) seeds (0/57) from each diseased plants.

Stability in crude sap: The thermal inactivation point lied between 60–65°C for 10 min exposures, the dilution end point is 10^{-4} – 10^{-5} , and the virus retained infectivity for more than 6 days at 20°C.

Table 2. Beetle transmission tests of a strain of BRMV

Beetle	Diseased plant for acquisition ^{a)}	Inoculated plant ^{a)}	No. of infected/inoculated plants
<i>Ceratoma</i> sp.	<i>P. vulgaris</i>	<i>P. vulgaris</i>	4 / 5
<i>Ceratoma</i> sp.	<i>P. vulgaris</i>	<i>G. max</i>	2 / 5
<i>Diabrotica</i> sp.	<i>P. vulgaris</i>	<i>P. vulgaris</i>	3 / 5

a): Acquisition and inoculation access periods were 2 days and 1 day, respectively.

Ten beetles on 5 plants were used in each trial.

Electron microscopy: Isometric particles with about 30 nm in diameter were detected in the partially purified preparation. In ultrathin sections, isometric virus-like particles forming crystal-like arrays were observed, many of which were in hexagonal arrays in the cytoplasm of infected French bean leaves (Plate 1).

Serology: In agar gel diffusion tests, the virus reacted positively with antiserum to the bean "mosaic-em-desenho" virus. The virus also reacted weakly with antiserum to cowpea severe mosaic virus (CpSMV), and a spur was observed between the precipitation line of a strain of BRMV and CpSMV (Plate 2).

Discussions

On the basis of host range, symptomatology, transmission, electron microscopy and serological affinities, the isolated virus from soybean was identified as a strain of BRMV, which is called bean "mosaic-em-desenho" in Brazil, a member of comovirus group⁶⁾.

It was reported that two species of beetles, *Cerotoma arcuata* and *Diabrotica speciosa* failed to

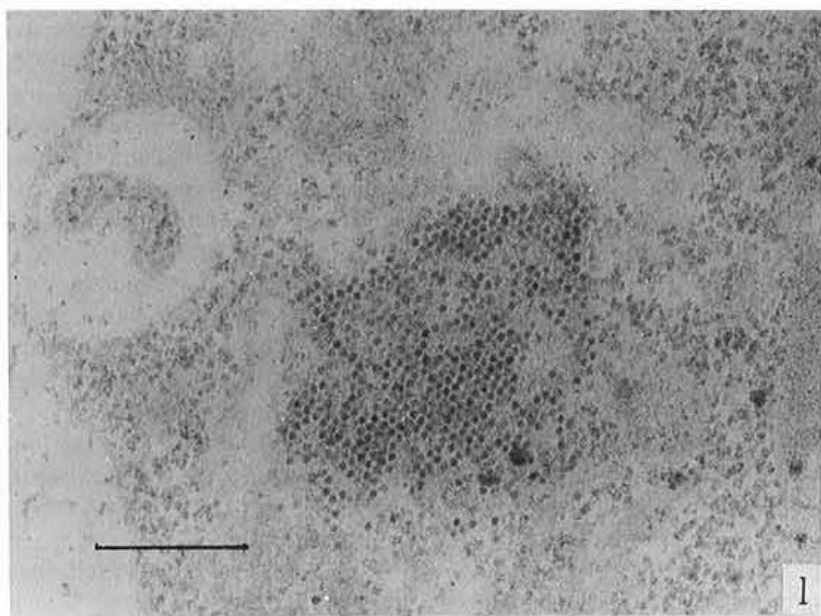


Plate 1. Crystal-like arrays of virus-like particles in the cytoplasm of infected French bean leaf
Bar presents 500 nm.

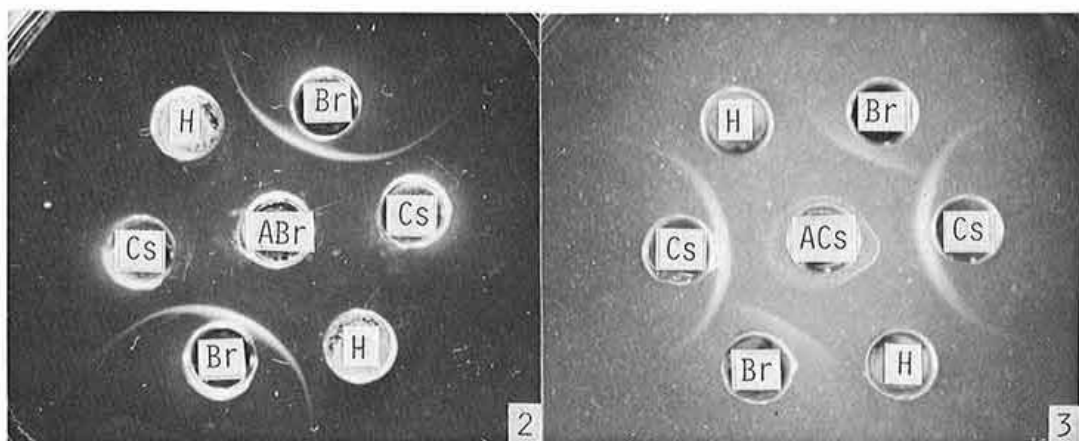


Plate 2. Serological relationship between the strain of BRMV and CpSMV to antisera against bean "mosaico-em-desenho" virus or CpSMV in agar gel diffusion tests

The center wells contained antiserum to well ABr: bean "mosaico-em-desenho" virus (left), and well ACs: CpSMV (right).

The peripheral well Br: a strain of BRMV from French bean plants, Cs: CpSMV from cowpea plants, and H: a healthy sap from French bean or cowpea plants.

transmit bean "mosaico-em-desenho" virus⁵). In the authors' transmission tests, however, the isolated virus from soybean was transmitted by those two species of beetles. It seems that the viruliferous beetles came from French bean fields to soybean fields. Although this virus is one of the most prevalent in French bean fields in Central Brazil, the natural occurrence of this virus on soybean has not been reported yet. An infected soybean plant with the virus might be difficult to detect in fields, because it usually shows very mild symptoms.

Incidences of BRMV have been reported in Costa Rica, El Salvador and Guatemala, where they are not economically important. Most of bean cultivars grown in Central America are resistant to BRMV⁴). The Colombian isolate of BRMV seems to be closely related to the Costa Rica strain, but it is slightly different from the El Salvador strain¹). In Brazil, however, it was reported that only one variety showed symptomless among the 168 bean varieties inoculated²), and 24 varieties showed symptoms among the 39 soybean varieties inoculated³). According to the study results obtained by the authors, no variety showed resistance among the 18 bean and 29 soybean varieties tested. From the results of testing on reactions of French bean varieties, it is very likely that the bean "mosaico-em-desenho" virus in Brazil is a different strain of BRMV which is identi-

fied in Central America. In order to establish an effective method for controlling this virus disease, further studies, etiological and epidemiological, are required in Central Brazil.

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