

# ATTEMPTS OF MECHANICAL TRANSMISSION AND SEROLOGICAL TESTS OF BEAN GOLDEN MOSAIC VIRUS IN BRAZIL

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## Introduction

Bean golden mosaic is a common disease that attacks bean and lima bean in Latin America. The causal virus is transmitted persistently in nature and in the laboratory by the whitefly, *Bemisia tabaci*. The disease was first reported in Brazil in 1965 (1) as a minor disease of bean in the State of São Paulo. In the last few years, it has become a major disease of bean and is now a limiting factor hampering bean production in Brazil. Attempts to transmit Brazilian bean golden mosaic virus (BGMV-B) by mechanical inoculation have failed to enhance the transmission (1, 2, 3).

In this paper, we describe the mechanical transmission and serological specificity of BGMV-B, Puerto Rican BGMV (BGMV-P) and mung bean yellow mosaic virus (MYMV) from Thailand.

## Materials and Methods

### 1. Virus source and maintenance

Bean and lima bean plants showing golden mosaic symptoms were collected from fields in EMBRAPA/CPAC, Núcleo Rural Rio Preto and Jardim D.F. in 1989. The virus was maintained in bean and lima bean plants by graft transmission in a greenhouse at CPAC, Brazil.

### 2. Mechanical inoculation

The inoculum of BGMV-B was prepared by grinding systemically infected young leaves in 0.1 M phosphate buffer, pH 7.8, containing 0.1% thioglycolic acid, using a chilled mortar and a pestle. Inoculations were

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performed by rubbing carborundum-dusted primary leaves of the test plants with cotton wool soaked in the homogenate. Seedlings of the test plants used for the mechanical inoculation tests were about 8 days old. The plant species tested were *Phaseolus lunatus* L. var. Henderson Bush (lima bean), *P. vulgaris* L. var. Carioca, Rico 23, Roxo and Top Crop (bean), *Glycine max* (L). Merr. var. Shirotsurunoko, and *Vigna radiata* var. M7A (mung bean).

### 3. Serological tests

Serological relationships among BGMV-B, BGMV-P and *Euphorbia* mosaic virus (EMV) were examined by agar gel double diffusion tests using 0.85% agar containing 0.8% sodium chloride and 1 mM sodium ethylenediaminetetraacetate (EDTA). All the antigens were partially purified from BGMV-B-infected leaves of Carioca bean and lima bean, and EMV-infected leaves of *Euphorbia brasiliensis* collected at CPAC. Healthy leaves of Carioca bean and *E. brasiliensis* were used as controls.

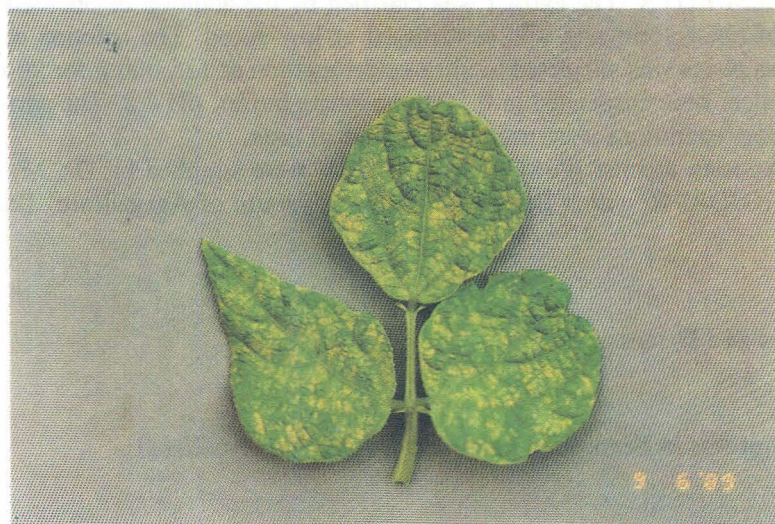
Fresh leaves were homogenized in 0.1M phosphate buffer, pH 7.8, containing 0.1% thioglycolic acid, 10 mM sodium diethyldithiocarbamate and 1 mM EDTA. The extract was clarified by the addition of one-half volume of chloroform and was centrifuged at low speed. The solution of the aqueous phase was subjected to ultra-centrifugation at 125,000 g for 90 min. The pellets were suspended in 0.1 M phosphate buffer, pH 7.8. The partially purified preparations were used for serological tests.

Antisera against BGMV-P and MYMV were supplied by Dr. M. Ikegami, NODAI Research Institute, Tokyo University of Agriculture, Tokyo, Japan.

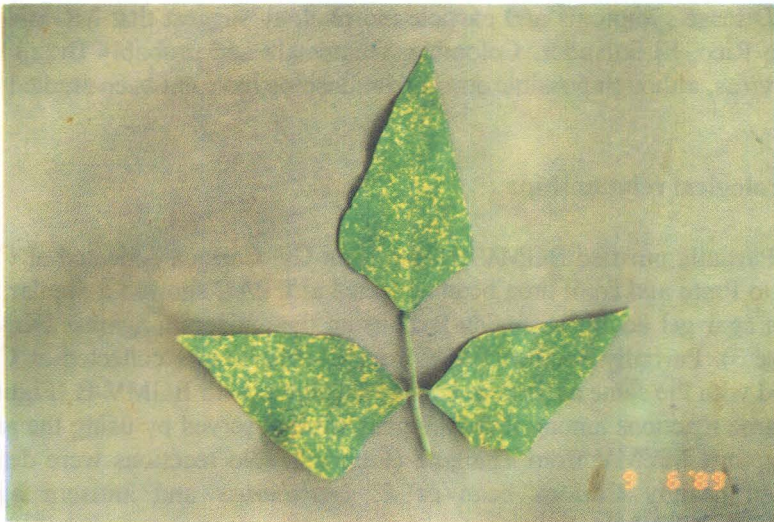
## Results and Discussion

### 1. Symptoms in bean, lima bean and *Euphorbia brasiliensis*

Carioca bean plants showing vein yellowing and golden mosaic symptoms were observed in the fields of CPAC. (Figure 1), Núcleo Rural Rio Preto and Jardim. Lima bean plants showing vein yellowing and golden mosaic symptoms in the fields of CPAC were grafted to healthy lima bean seedlings.



**FIG. 1 - Symptoms of bean golden mosaic disease in Carioca bean in the field of CPAC (Upper). An enlargement view of upper leaves of Carioca bean (Lower).**



**FIG. 2 - Symptoms of bean golden mosaic disease in grafted lima bean (Upper). An enlargement view of upper leaves of lima bean (Lower).**

Similar symptoms appeared in the grafted plants (Figure 2). *E. brasiliensis* plants showing vein yellowing symptoms were also found at the campus of CPAC and in the bean fields of Núcleo Rural Rio Preto and Jardim. The symptoms induced by BGMV-B in bean and lima bean were similar to those caused by BGMV-P (4, 5), but not to those caused by MYMV (6, 7).

## 2. Mechanical inoculation

Among the four plant species belonging to the family Leguminosae, no plants showed any symptoms after mechanical inoculation with the crude sap extracted from BGMV-B-infected lima bean leaves.

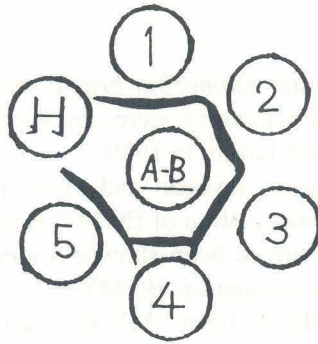
In Brazil many attempts made by several investigators to transmit BGMV-B by mechanical inoculation have failed (1, 2, 3). Our first attempt of transmission by mechanical inoculation of BGMV-B was also unsuccessful in a greenhouse at CPAC, Brazil. We have reinoculated BGMV-B mechanically to several legumes. It is necessary to observe whether the symptoms will appear in the inoculated plants.

Disease symptoms and particle morphology suggest that MGMV from Puerto Rico, El Salvador, Colombia, Guatemala and probably Brazil is the same virus, although possible strain relationships have not been studied (4).

## 3. Serological relationships

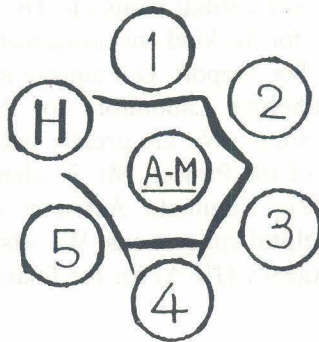
Partially purified BGMV-B from bean Cv. Carioca collected at CPAC and Rio Preto and from lima bean collected at CPAC showed a similar reaction in agar gel double diffusion tests using the antiserum against BGMV-P (Figure 3). Partially purified EMV from *E. brasiliensis* collected at CPAC reacted with the same antiserum by forming spurs with BGMV-B (Figure 3). The same reactions among these antigens were observed by using the antiserum against MYMV from Thailand (Figure 4). No reactions were detected between healthy Carioca bean of *E. brasiliensis* and antisera against BGMV-P or MYMV.

It thus appears that there is no serological difference between BGMV-B and BGMV-P on the basis of serological tests. BGMV-B could be distinguished from EMV by the use of antisera against BGMV-P or MYMV. BGMV-B was different from EMV serologically.



**FIG. 3 - Agar gel double diffusion tests of Brazilian bean golde mosaic virus (BGMV -B) and *Euphorbia* mosaic virus (EMV).**

A-B= Antiserum against Puerto Rican BGMV. - 1= BGMV-B from Carioca bean in CPAC. - 2,5=BGMV-B from lima bean in CPAC. - 3=BGMV-B from Carioca bean in Rio Preto. - 4=EMV from *Euphorbia* in CPAC.



**FIG. 4 - Agar gel double diffusion testes of Brazilian bean golden mosaic vírus (BGMV-B) and *Euphorbia* mosaic virus (EMV).**

A-M= Antiserum against mung bean yellow mosaic virus. - 1= BGMV-B from Carioca bean in CPAC. 2,5=BGMV-B from Carioca bean in CPAC. - 3=BGMV-B from Carioca bean in Rio Preto. - 4=EMV from *Euphorbia* in CPAC.

## Conclusion

1. Bean, lima bean and *Euphorbia brasiliensis* plants showing golden mosaic or vein yellowing symptoms were observed in the fields of CPAC, Núcleo Rural Rio Preto and Jardim in 1989.

2. No bean, lima bean, soybean and mung bean plants showed any symptoms by mechanical inoculation of BGMV-B from infected lima bean.

3. BGMV-B from Carioca bean showed a identical reaction in serological tests using the antiserum against BGMV-P.

4. BGMV-B was different from EMV serologically.

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We have conducted some experiments on bean golden mosaic virus of bean and lima bean. The results obtained are described in the present paper.

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