

## Inclusion bodies induced by *Bean rugose mosaic comovirus* seen under light microscopy

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**Abstract:** Two types of inclusion bodies were consistently observed under light microscopy in bean (*Phaseolus vulgaris*) leaf tissue infected with *Bean rugose mosaic virus* (BRMV), a species of the genus *Comovirus*, family Comoviridae. One type consisted of vacuolated inclusions found mainly in the cytoplasm of epidermal cells. The other type consisted of abundant crystalloid inclusions of different sizes and shapes found consistently in glandular hairs, guard cells, phloem tissue, xylem elements and occasionally in epidermal and mesophyll tissues. The two types of inclusion bodies stained with Azure A and Luxol Brilliant Green BI-Calcomine Orange 2RS (O-G), and were similar to those seen to be caused by other species of comoviruses.

**Key words:** *Comovirus*, cytopathology, light microscopy, diagnosis, bean.

*Bean rugose mosaic virus* (BRMV) belongs to the genus *Comovirus* and to the family Comoviridae of plant viruses. It was first described infecting beans (*Phaseolus vulgaris*, L) in Costa Rica (Gómez 1972, 1982); it also occurs in Guatemala (Cartín 1973), El Salvador (Gálvez *et al.* 1977), Brazil (Lin *et al.* 1981), Colombia (Acosta *et al.* 1986), Honduras and Nicaragua (Gómez 1977). In bean, it causes a severe mosaic, rugosity and deformation of leaves and pods, and in some cases severe stunting and blistering of leaves. Its host range is restricted to the species *Phaseolus vulgaris* and *Vicia faba*, and *Chenopodium amaranticolor* of the Leguminosae and Chenopodiaceae families respectively, and it is transmitted by leaf-feeding beetles (Gómez 1972, 1982).

Fifteen species have been identified for the *Comovirus* genus (Murphy *et al.* 1995), eight of which have been studied cytologically.

All comoviruses studied thus far induce the formation of two different types of cytoplasmic inclusion bodies: a) crystalline inclusions of various and unusual shapes composed of viral particles, which stain violet in Azure A and green in the OG combination (Luxol Brilliant Green BL-Calcomine Orange 2 RS), and are frequently observed in glandular hair cells, guard cells and phloem tissue and occasionally in the epidermis and mesophyll; and b) vesiculate-vacuolate inclusions observed in the cytoplasm of epidermal cells which stain green in OG and red to magenta in Azure A (Gibbs and Paul 1970, Kim and Fulton 1972, Kitajima *et al.* 1974, Langenberg and Schroeder 1975, Bruening 1978, Edwardson and Christie 1991). Both types of inclusion bodies and their location are considered to be main diagnostic characteristics of the *Comovirus* genus (Edwardson and Christie 1978). Xylem elements blocked with masses

of virus particles have also been observed in *Squash mosaic virus* and *Cowpea mosaic virus* infections (Christie and Edwardson 1977). In this paper, inclusion bodies observed by light microscopy in BRMV-infected bean leaf tissue are described and compared with those induced by other members of the genus *Comovirus*.

#### MATERIALS AND METHODS

**Virus isolate:** the isolate of BRMV was initially obtained from plants of *P. vulgaris* infected in the field and later maintained in the greenhouse by mechanical inoculation on bean plants. Leaves from plants showing severe mosaic and leaf deformation, and stunting and blistering of leaves and pods (Fig. 1) were

selected for examination by light microscopy. Healthy leaves from the same bean variety were also examined.

**Virus identification:** the identity of the virus in all plants selected for light microscopy was confirmed by DAS-ELISA using rabbit polyclonal antibodies produced by C. Rivera (1990).

**Light microscopy:** strips from the lower epidermis of the leaves of inoculated and non-inoculated bean plants were removed and stained in 0.1% Azure A and OG (Christie and Edwardson 1977). After removal of the epidermis, the mesophyll was soaked in 2-methoxyethanol for 30 min to remove the chlorophyll and stained with Azure A. The excess stain was removed in 95% ethanol. The tissue was mounted directly in Euparal and observed with a light microscope and photographed.



Fig. 1. Symptoms induced by *Bean rugose mosaic comovirus* mechanically inoculated in the greenhouse to leaves of *Phaseolus vulgaris*. Systemically infected plants showed mosaic, deformation, stunting and blistering of leaves.

Síntomas inducidos por el virus del mosaico rugoso del frijol (comovirus) inoculado mecánicamente en hojas de *Phaseolus vulgaris*. Las plantas con infección sistémica mostraron mosaico, deformación, enanismo y ampollamiento en las hojas.

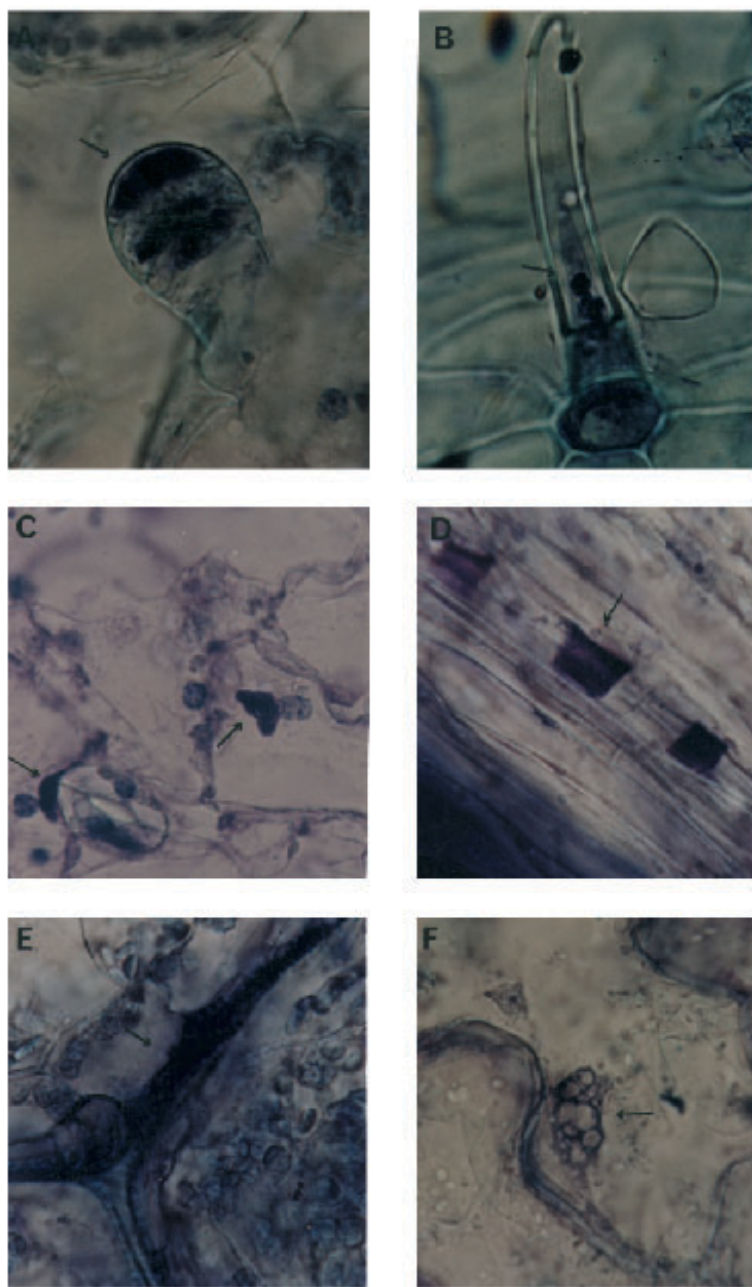


Fig. 2. Inclusion bodies in leaf tissue infected with *Bean rugose mosaic comovirus* stained with Azure A and observed under light microscopy. Crystalline inclusions observed in: (A) glandular hair cells, (B) trichome cells, (C) guard cells and epidermis, (D) phloem tissue and (E) xylem elements. Vesiculate-vacuolate inclusions observed in: (F) cytoplasm of epidermal cells. Arrows indicate the presence of inclusion bodies.

Inclusiones en el tejido foliar infectado con el virus del mosaico rugoso del frijol (comovirus), teñidas con Azure A y observadas al microscopio de luz. Inclusiones cristalinas observadas en: (A) células de un tricoma glandular, (B) células de tricoma, (C) células guarda y epidermis, (D) tejido del floema y (E) elementos del xilema. Inclusiones vesiculadas-vacuoladas observadas en: (F) citoplasma de células de la epidermis. Las flechas indican la presencia de los cuerpos de inclusión.

## RESULTS

Two types of inclusion bodies were consistently observed by light microscopy in bean leaf tissue infected with *Bean rugose mosaic comovirus*. Crystalline cytoplasmic inclusions of different size and shape were frequently found in glandular hair cells (Fig. 2 A), trichome cells (Fig. 2 B), epidermis and guard cells (Fig. 2 C) and phloem tissue (Fig. 2 D). The xylem elements observed were blocked with masses of crystalline inclusions (Fig. 2 E). Vesiculate-vacuolate inclusions were also frequently found in the cytoplasm of epidermal cells (Fig. 2 F). Both types of inclusions stained with Azure A and OG combination.

## DISCUSSION

All comoviruses studied to date are reported to induce vacuolate-vesiculate inclusions and crystalline inclusions of virus particles. Both types of inclusion bodies are now considered to be main characteristics of the *Comovirus* genus (Edwardson and Christie 1991). Vacuolate-vesiculate inclusions in epidermal cells and crystalline inclusions of virus particles have been described in trichome cells, spongy parenchyma, phloem, mesophyll cells and xylem elements by electron microscopy of BRMV infected leaves (Kitajima *et al.* 1974, Gálvez *et al.* 1977, Gámez 1982, Muñoz and Kitajima 1990). Both types of inclusion bodies were easily identified in this research by light microscopy of BRMV-infected tissue stained with Azure A and with O-G. Thus light microscopy should be useful in the diagnosis of BRMV infections.

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## RESUMEN

Se observaron dos tipos de inclusiones virales, mediante microscopía de luz, en hojas de plantas de frijol (*Phaseolus vulgaris*) previamente infectadas con el virus del mosaico rugoso del frijol ("*Bean rugose mosaic virus*", BRMV), especie del género *Comovirus*, familia Comoviridae. Se hallaron inclusiones vesiculadas, principalmente en el citoplasma de células de la epidermis, y abundantes inclusiones cristalinas de diferentes formas y tamaños siempre en células guarda, tricomas glandulares, floema, elementos del xilema y ocasionalmente en células epidérmicas y del mesófilo. Ambos tipos de inclusiones tiñeron con Azure A y con la tinción, verde naranja (Luxol Brilliant Green BL-Calcomine Orange 2 RS) conocida como OG, y son similares a las inclusiones inducidas por otras especies del género *Comovirus*.

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