

## Incidence and Characterization of Bean Common Mosaic Virus Isolates in Spanish Bean Fields

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### ABSTRACT

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A 5-yr survey for the presence of bean common mosaic potyvirus (BCMV) in the major bean (*Phaseolus vulgaris*) growing areas of Spain was conducted, and the viral isolates were characterized biologically and serologically. Both serotype A and B were found. Necrosis-inducing serotype A isolates, found for the first time in Spain, resembled NL-3. Serotype B isolates resembled NL-4 and US-5. The complex antigenic relationships of Spanish BCMV isolates, as determined with specific monoclonal and polyclonal antibodies, and the incidence and distribution of serotypes are discussed.

Common bean (*Phaseolus vulgaris* L.) is one of the most widely grown protein-producing food legumes. It is extensively cultivated in South and Central America, and also in eastern Africa, eastern Asia, North America, and western and south-eastern Europe. In Spain, 122,109 ha were grown in 1988, with a production of 318,897 t. Dry bean production is concentrated in northwestern Spain, and fresh bean production in the south-eastern region of the country.

The susceptibility of bean to viruses is a major yield-reduction factor worldwide. At least 34 viruses are reported to infect bean naturally. Some of these viruses are seedborne and most are transmitted by insects (7).

There has been no previous report on the incidence of viruses in bean in Spain, although their presence was assumed because of low yields and viruslike symptoms observed. To determine which viruses occur in Spanish bean fields, a preliminary survey was conducted in July 1989 and August 1990. In this limited survey, bean common mosaic potyvirus (BCMV) was the virus most frequently found. The virus has worldwide distribution, is seed transmitted, and is readily spread by aphids in a nonpersistent manner (10). Many strains of BCMV have been differentiated by symptoms produced on a selected group of bean cultivars (3). Strains are classified in two main groups: 1) the mosaic-inducing strains, and 2) the temperature-insensitive, necrosis-inducing strains, which

cause systemic vascular necrosis in bean genotypes possessing the dominant *I* gene. These biologically different groups of strains are serologically distinct and are described as serotypes B and A, respectively (9,14). Analysis of the coat protein genes and 3' noncoding regions of different BCMV isolates revealed complex relationships among BCMV strains and other potyviruses that infect legumes (8,12,13), and a BCMV subgroup comprising at least two different potyviruses has been proposed (2).

This work describes the incidence and distribution of BCMV in Spain from 1989 to 1993 and the serological and biological characterization of some Spanish BCMV isolates.

### MATERIALS AND METHODS

**Sample collection.** From 1989 to 1993, leaves of bean showing viral disease symptoms were collected from 11 bean-growing regions (Fig. 1). In a preliminary survey, 30 samples were collected from three central and northwestern areas in the summers of 1989 and 1990. A larger survey was conducted from 1991 to 1993 covering the main regions where common bean is grown in Spain (Fig. 1). Fields in the northwestern part of the country were usually nonirrigated. Beans in the central and southwestern regions were frequently grown in plastic greenhouses.

**Virus identification.** Potyviral infections and BCMV serotypes were identified by indirect enzyme-linked immunosorbent assay (ELISA) (6). Samples were extracted in 0.05 M carbonate buffer, pH 9.6, containing 1% polyvinylpyrrolidone-40 (PVP-40), and tested with the antibodies listed below. Reagents used, other than the substrate, were

diluted in phosphate-buffered saline containing 0.1% Tween 20 and 2% PVP-40. Alkaline phosphatase-labeled goat anti-mouse antiserum (for monoclonals) was used at a dilution of 1:2,000. The *p*-nitrophenyl phosphate substrate was used at a concentration of 1 mg/ml in diethanolamine buffer, pH 9.8. Absorbances at 405 nm were measured in a Titertek Multiscan Plus MK II ELISA reader (Labsystems Inc., Vancouver, CA). Reactions were considered positive when they were more than twice the absorbance value of healthy controls.

The monoclonal antibodies used in ELISA were 1) the anti-potyvirus group PTY (Agdia Inc., Elkhart, IN) at a dilution of 1:200, 2) the broad spectrum antibody for BCMV II-197 from G. Mink (Prosser, WA) (15) at a concentration of 0.1 mg/ml, 3) the BCMV serotype A-specific antibodies I-2 and I-3 from G. Mink at 0.1 mg/ml, and 4) the serotype B-specific antibody 15E5 from H. J. Vetten (Braunschweig, Germany) at a dilution of 1:50.

**Identification of BCMV strains.** Virus strains were identified by their reactions on differential bean cultivars (3). Virus-free seed of these cultivars were provided by F. J. Morales (Cali, Colombia) and R. Ortiz (Hispareco, S. A., Badajoz, Spain). Test plants were mechanically inoculated by rubbing primary leaves, previously dusted with 400-mesh Carborundum, with extracts of infected samples at a 1:10 (w/v) dilution in 10 mM phosphate buffer, pH 7.2. All plants were kept in an insect-free greenhouse (870  $\mu\text{E}\cdot\text{m}^{-1}\cdot\text{sec}^{-1}$ , 26 C). Symptoms were scored after 4 wk. Inoculated plants of genotype *ii* cultivars were analyzed by ELISA with serotype-specific monoclonal antibodies.

### RESULTS

More than 15 varieties and cultivars were collected, including determinate and indeterminate climbing growth habit types, and dry and fresh bean cultivars. The number of fields sampled varied from one to seven in each region. A total of 41 fields was surveyed.

Field symptoms consisted of green, yellow, and vein-banding mosaic; leaf malformation and rolling; and stunting. Most samples were collected from plants showing these symptoms. A sample from

a symptomless plant was collected from each field. Samples were put in plastic bags, labeled, and transported in an ice box.

**Virus incidence.** Potyviruses were commonly found in samples collected in 1989 and 1990, and 23 of the 30 samples (77%) reacted positively in ELISA with the PTY antibody. Of these 23, 70% also reacted with the broad spectrum monoclonal antibody for BCMV II-197. Other viruses identified on the basis of host range and ELISA (1) included three samples with cucumber mosaic virus (CMV) and one with bean yellow mosaic virus (BYMV).

Of the 264 samples collected between 1989 and 1993, 189 (72%) were infected

by potyviruses. A total of 95% (180) of these were infected with BCMV or closely related potyviruses.

**Serotype identification.** Table 1 records the incidence of BCMV serotypes A and B, and Figure 1 shows their geographical distribution. Serotype A isolates were detected in 5% of the samples (i.e., 12 of 234 samples), and serotype B isolates in 56% (130 of 234). Mixed infections of both serotypes were detected in 5% of the samples, and unknown potyviruses in 5.5% (Table 1).

All the BCMV-A isolates reacted with monoclonals PTY (anti-potyvirus group), II-197 (broad spectrum), and I-2 and I-3 (both A-specific). The B-specific monoclonal 15E5 did not react with any

of the BCMV-A isolates. No differences in reactivity were observed between A-specific monoclonals I-2 and I-3.

All of the BCMV-B isolates reacted with monoclonals PTY (anti-potyvirus group), II-197 (broad spectrum), and 15E5 (B-specific), but not with I-2 and I-3 (both A-specific).

Samples that reacted with monoclonals 15E5 (B-specific) and I-2 and I-3 (A-specific) were considered to be infected with isolates from both serotypes. All of these reacted with PTY (anti-potyvirus group), but three did not react with broad spectrum II-197.

Thirteen samples (5.5%) reacted with PTY (anti-potyvirus group) and II-197 (broad spectrum), but not with the BCMV-specific monoclonals. These were judged to contain other potyviruses closely related to BCMV.

**BCMV strain determination.** Nine BCMV isolates from two different regions in northwestern Spain (León and Pontevedra) were characterized biologically. Four of these were serotype B isolates and five were serotype A. All of the five BCMV-A isolates showed host reactions identical to those described for strain NL3 (3). One of the BCMV-B isolates was identified as strain NL4, and the three remaining as US5/Fla.

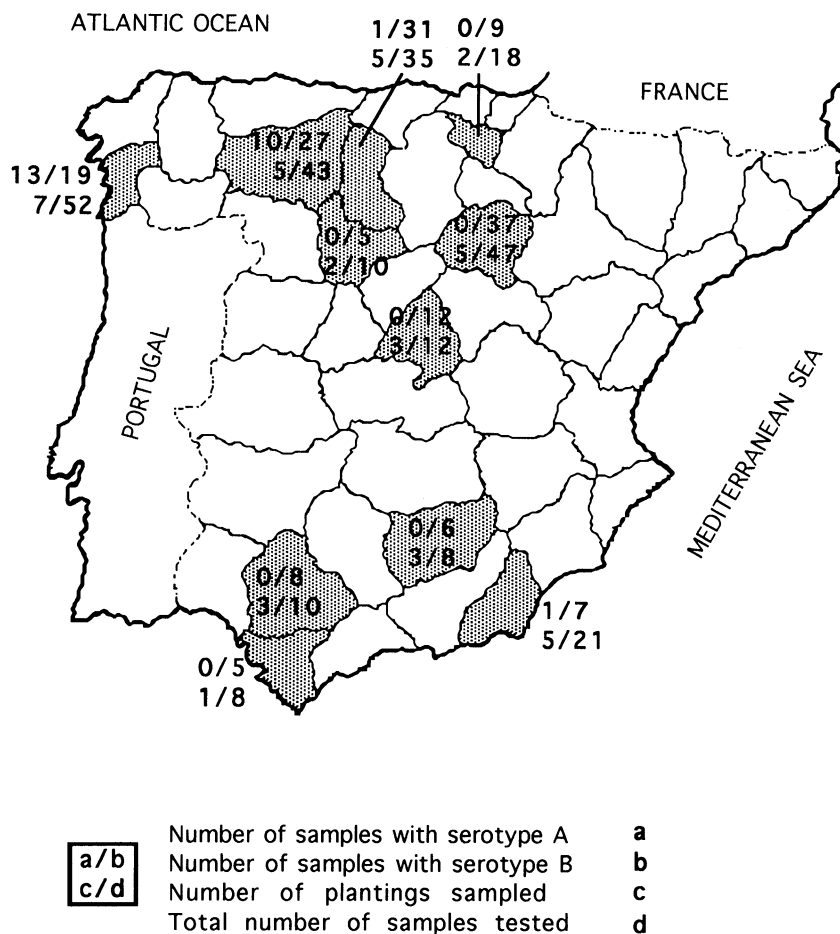
## DISCUSSION

Our results show that BCMV is the most prevalent potyvirus infecting bean in Spain, with 95% of the potyvirus-infected samples reacting with BCMV serotype-specific monoclonal antibodies. The high percentage (72%) of potyvirus infections in the samples collected is also remarkable. To our knowledge, this is the first report of BCMV-A in Spain.

Seedborne BCMV infections are known to serve as the primary inoculum source in bean fields for subsequent virus spread through nonpersistent aphid transmission (5). Commercial seed lots infected with BCMV are frequently detected in our laboratory, and serotypes A and B have been identified by ELISA. In some seed lots, the incidence of BCMV reached 40% (data not shown). Thus, it was not surprising that these viral strains were found in the bean-growing areas of Spain.

Knowledge of the strains present in the bean-growing regions is valuable for resistance breeding using available resistance genes. Our data from the biological characterization of selected BCMV isolates show that NL3 could be the prevalent necrosis-inducing strain in the regions surveyed.

The complexity of the reactions between BCMV isolates and the set of monoclonal and polyclonal antibodies used shows again the heterogeneity of BCMV isolates and strains, at least at the coat protein level. Significant differences in size and sequence between serotype A and B isolates have been



**Fig. 1.** Geographical distribution of bean common mosaic potyvirus serotypes across the regions surveyed (shaded areas) in mainland Spain.

**Table 1.** Number of samples assigned to each bean common mosaic potyvirus (BCMV) serotype, mixed infections, and other closely related potyviruses according to reactions against specific monoclonal antibodies

Viral isolate assignment	Number of positive samples <sup>a</sup>	Percent total BCMV-infected samples	Percent total potyvirus-infected samples
BCMV-A	12	8	7
BCMV-B	130	84	75
BCMV-A + B	12	8	7
Other potyviruses	13	...	8

<sup>a</sup>A total of 234 samples were tested from 1991-1993.

described (4,13), and while serotype A coat protein seems to be conserved at the amino acid level, serotype B isolates exhibit a major variability in the N-terminal region of the protein. These data are supported by HPLC results (8) and sequence data obtained in our laboratory (12).

A molecular approach to BCMV biology will help clarify the relationships among the serotypes, strains, and isolates of this potyvirus. We are currently working in molecular characterization of Spanish isolates and the improvement of viral detection in infected leaf and seed tissue (11).

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