



DETECTION OF BEAN-INFECTING VIRUSES IN CALIFORNIA WITH AN EMPHASIS ON THE CRSP-FACILITATED WORK

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INTRODUCTION

Worldwide, common bean (*Phaseolus vulgaris* L.) is infected by numerous viruses, but most of these are not transmitted via seed (Hall, 1991). However, *Bean common mosaic virus* (BCMV) and the closely related *Bean common mosaic necrosis virus* (BCMNV) are two bean-infecting potyviruses that are seed-transmitted and are widely distributed in bean crops throughout the world (Galvez and Morales, 1989). As typical members of the Family *Potyviriidae* (potato virus Y family of viruses), BCMV and BCMNV possess long flexuous rod-shaped virions (~750X15 nm), and both viruses are transmitted plant-to-plant by various species of aphids. Because BCMV and BCMNV can also be transmitted in bean seed at high rates and then can cause significant yield losses in heavily infected fields, they are of significant concern for the production of high quality bean seed. An additional complexity is that the dominant *I* resistance gene, which confers high levels of resistance to BCMV, conditions a systemic hypersensitive death (also referred to as black root rot) in bean plants inoculated with BCMNV. Furthermore, in a non-*I* gene variety, symptoms induced by BCMV and BCMNV are indistinguishable. Thus, in areas where BCMNV is endemic, such as Africa, the *I* gene has limited usefulness due to the potential widespread death of plants during epidemic outbreaks of BCMNV. In the United States, BCMV is endemic whereas BCMNV is not endemic, and when BCMNV does appear, it is usually introduced via seed of a non-*I* gene variety.

California is a leading producer of certified bean seed. This is in large part due to the fact that California has a dry environment during the growing season, which is not conducive to the development of bacterial and fungal diseases. However, in some years BCMV can be a major problem for bean seed production in California, and BCMNV represents an exotic virus that needs to be kept out of California. There are also a number of other viruses that infect beans in California, including *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), and *Alfalfa mosaic virus* (AMV). All these viruses pose significant potential problems for the California bean seed industry and they can be difficult, if not impossible, to identify based upon symptoms alone.

BEAN VIRUS DETECTION AND CONTROL

BCMV can be effectively controlled through the use of certified seed and/or resistant varieties (Galvez and Morales, 1989; Hall, 1991). Because resistant varieties are not presently available for all bean classes produced in California, it is necessary to include BCMV monitoring in the seed certification process. In this process, fields are visually inspected by personnel working for the California Crop Improvement Association (CCIA), and fields having more than 0.5% BCMV are rejected for certification. However, in the field, symptoms induced in beans by BYMV, CMV and AMV, which are generally not seed-transmitted in common bean, can be confused with

symptoms of BCMV. In addition, symptoms of BCMV can be masked if plants are inspected at older stages of growth. Thus, it is important to have the capability to differentiate BCMV from other non-seed-transmitted viruses and to have methods that allow for the sensitive and reliable detection of BCMV and BCMNV.

Thus, in the early 1990's, a project was initiated as part of the Bean/Cowpea CRSP and in conjunction with CCIA to develop improved methods for detecting bean-infecting viruses. In this program, we work in close conjunction with CCIA personnel to provide an accurate identification of bean-infecting viruses observed in the field. The ability to detect BCMNV, which can not be distinguished from BCMV in non-I gene varieties (BCMNV results in a systemic hypersensitive reaction in I gene varieties resulting in the death of infected plants), provides the capacity for rapid detection of this virus if it were inadvertently introduced to California. Establishment of BCMNV in California would be a major problem for the industry because of the emphasis on introducing the *I* gene into bean cultivars for BCMV resistance. Thus, the CCIA/UCD/Bean/Cowpea CRSP bean-infecting virus detection program serves as a first line of defense to keep BCMNV out of California.

In this project, a number of tools are used to detect bean-infecting viruses, and these vary depending on the virus and the type of tissue being tested. However, the key technique that is utilized is serology and, more specifically, the enzyme-linked immunosorbent assay (ELISA). For leaf tissue, sap inoculation of indicator plants also may be used and for seed, grow-out tests may be used. As part of an inter-CRSP collaboration, Dr. Gaylord Mink of the then WSU-Tanzania project, provided us with monoclonal antibodies (McAbs) that allow for the detection and differentiation of BCMV (BCMV B serotype) and BCMNV (BCMV A serotype). These McAbs were: McAb 197, which detects all BCMV strains, and McAb I-2, which detects only BCMNV. Additional antisera were obtained for BYMV (polyclonal antibody 204-1 from Dr. O. W. Barnett, North Carolina State University), CMV (various sources) and AMV (Dr. Mike Davis, UC Davis).

Beginning in 1991, CCIA personnel provided leaf samples from plants suspected to have virus infection and these were tested by ELISA for five viruses (BCMV, BCMNV, BYMV, CMV and AMV). Early on in the testing of samples (1993), it was realized that the predominant bean-infecting viruses were BCMV and BYMV, with CMV and AMV being relatively rare and sometimes not even detected in a given year ([Table 1](#)).

Thus, we generally focused upon BCMV, BCMNV and BYMV. A summary of virus detection results for bean seed production fields in California from 1991 to 2000 is presented in [Table 1](#). These results indicate that BCMV was the most prevalent bean-infecting virus in California over this testing period. The variability in the incidence of BCMV can be attributed to fluctuations in the aphid vector populations in a given year as well as the amount of non-I gene bean varieties submitted for certification in a given year. What is clear is that BCMV can reach high incidences in a given year and that there are still many non-I gene bean varieties being grown in California. Thus, it is important to include the virus identification portion of the field inspection in order to keep the incidence of BCMV in seed at a relatively low level. The next most prevalent virus detected in this program was BYMV. The incidence of BYMV also varied considerably from year-to-year, presumably due to the populations of aphids as well as the type and location of bean plantings. The greatest concern about BYMV is that it's visual symptoms can be confused with BCMV and, because BYMV is not seed-transmitted, this could result in the improper rejection of a field for certification. In 2000, an unusually high incidence of CMV was detected; this demonstrated the year-to-year fluctuation that can be seen among bean-infecting viruses and the need for precise testing methods for identification of various bean-infecting viruses.

Table 1. Detection of *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), and *Alfalfa mosaic virus* (AMV) in bean leaf samples collected from bean seed production fields in the California Crop Improvement Association seed certification program/UC Davis/Bean/Cowpea CRSP bean-infecting virus detection program.

Year	Number of samples	No. Samples Infected with				
		BCMV	BCMNV	BYMV	CMV	AMV
1991	42	28	-- ^a	2	nt ^b	nt
1992	71	30	--	7	8	1
1993	588	363	--	147	3	1
1994	76	49	--	5	nt	nt
1995	133	100	--	5	nt	nt
1996	106	56	18	2	nt	nt
1997	389	242	--	5	nt	nt
1998	82	39	--	3	nt	nt
1999	474	215	--	54	nt	7
2000	279	62	--	--	80	nt

^a--=Not detected

^bnt=Not tested

OUTBREAK OF BCMNV IN CALIFORNIA

During the 1996 growing season, an outbreak of BCMNV was detected in a navy bean field (cv. Snow Bunting) in Colusa County, California. ELISA tests performed on leaves from these fields revealed a positive result with both the 197 and I-2 McAbs, suggesting the presence of BCMNV. Inoculation of indicator bean plants confirmed the presence of BCMNV and suggested it was the NL-3 strain of BCMNV. To further characterize this BCMNV isolate, reverse transcriptase PCR (RT-PCR) was used to amplify a portion of the genome of the virus that contains the 3' end of the capsid protein (CP) gene and the non-translated region (UTR). An approximately 650 bp fragment was amplified, and DNA sequence analysis revealed that the nucleotide sequences of the 3' end of the CP and the UTR were 98% and 94% identical to those of a Michigan isolate of BCMNV NL-3, respectively. Together, these results suggested that the outbreak of bean common mosaic in this field was due, at least in part, to the NL-3 strain of BCMNV (Guzman et al., 1997). After BCMNV was identified in this field, beans were harvested for food purposes and were not used for seed. The following season, legume fields in the area around this field were surveyed for bean common mosaic symptoms. No evidence was found of BCMNV in the 1997 or subsequent growing seasons, suggesting that this outbreak of BCMNV was contained. This is a very good example of the value of the CCIA/UC Davis/Bean Cowpea CRSP virus detection program for identifying and containing outbreaks of new viruses.

OUTBREAK OF CLOVER YELLOW VEIN VIRUS IN CALIFORNIA

In 1999, a severe outbreak (i.e., 100% infection) of a virus disease was observed in a single bean field in Colusa County, California. The symptoms included a yellow mosaic, leaf epinasty and, in some plants, a systemic necrosis. This field was adjacent to a clover field that had been harvested early in the development of the bean plants. A preliminary serological test (ELISA) suggested that the virus infecting these bean plants was *Peanut mottle virus* (PeMoV). This would have represented the first report of this virus in California. A range of bean cultivars (cvs. Black Turtle Soup, Topcrop, California Early Light Red Kidney and Sutter Pink) were inoculated with sap prepared from symptomatic leaves collected from this field. Symptoms developing on

these plants ranged from systemic necrosis (cvs. Sutter Pink and Black Turtle Soup) to strong yellow green mosaic and leaf distortion (cvs. Topcrop and California Early Light Red Kidney). Furthermore, inoculated primary leaves of cv. Topcrop failed to develop local lesions, which is characteristic of PeMoV. ELISAs were performed with leaves of all symptomatic plants with antisera against PeMoV, BYMV, BCMV and BCMNV, as well as RT-PCR analysis with primer pairs specific for PeMoV, BYMV, BCMV and BCMNV. All of these tests were negative.

To further investigate the nature of this virus, a minipurification method was used to purify virions from symptomatic leaves of all four cultivars. SDS-PAGE analysis of purified virions from these cultivars revealed a 32 kDa band consistent with infection by a potyvirus. Transmission electron microscopy analysis of these preparations revealed the presence of potyvirus-like flexuous rods (approximately 750 nm long and 15 nm wide). We next designed a primer pair specific for the coat protein gene of *Clover yellow vein virus* (CIYVV), and RT-PCR with these primers resulted in the amplification of a 630 bp DNA fragment from four isolates of this potyvirus. No fragments were amplified from an uninfected control. The PCR-amplified fragments were direct-sequenced, and sequence comparisons revealed that the sequences of all four isolates were 95% identical to that of CIYVV (Genbank accession number D89541). Subsequently, a CIYVV antiserum was obtained from Dr. Simon Scott, (Department of Plant Pathology, Clemson University), and ELISAs performed on leaves infected with all four isolates were positive. Finally, to assess whether the virus was seed-transmitted, seed harvested from this field was planted in a greenhouse (two lots of 400 seed each). None of the plants from these seeds developed virus symptoms, suggesting that the virus was not seed-transmitted. Together, these results indicated that the virus disease outbreak in this bean field was caused by CIYVV rather than PeMoV. The inoculum source for the virus was probably the adjacent clover field. This is the first report of CIYVV infecting beans in California (Crnov and Gilbertson, 2001).

CONCLUSIONS

The bean-virus detection program represents an example of one of the benefits that Bean/Cowpea CRSP research has had for U.S. bean production. The bean seed industry in California has been very appreciative of this program and has provided some funding for this work. In addition, Bean/Cowpea CRSP scientists have received training in ELISA and other virology techniques at the UC-Davis facility. While a long-term goal of the industry is to develop BCMV resistant bean varieties of all the major market classes grown in California through incorporation of the *I* gene, the industry will need to be more vigilant than ever to keep BCMNV from becoming established in California. Alternatively, the *I* gene can be 'protected' from inducing the systemic necrosis upon infection by BCMNV by incorporation of certain recessive genes (e.g., *bc-3* gene), but this is challenging from a breeding perspective and will require extensive breeding efforts.

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