

## Distribution Pattern of Bean Common Mosaic Virus in Developing Bean Seed

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### ABSTRACT

The distribution of bean common mosaic virus (BCMV) in developing reproductive tissues was determined using 'Monroe' bean for local lesion assays. Opened and unopened blossoms, as well as young pods (and enclosed seeds) contained infectious BCMV. BCMV was detected internally in cotyledons and embryos, but not in seedcoats of seeds developing on infected plants; BCMV was easily removed from seedcoat surfaces by external decontamination. Seed maturation (drying) had little effect on BCMV distribution in cotyledons and embryos.

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Bean common mosaic virus (BCMV) remains one of the serious pathogens of bean (*Phaseolus vulgaris* L.) wherever susceptible varieties are grown. Seed transmission undoubtedly plays an important role in the epidemiology of this disease, and is primarily responsible for long-distance dissemination of the virus (8). In recent yr, numerous new strains of BCMV have been described (6), and the threat of seed transmission of such strains to commercial bean production areas of the U.S. is real.

Although there are numerous reports of seed transmission in BCMV, little information is available regarding the mechanism of seed infection and transmission. This lack of information may be caused partly by the lack of a reliable local-lesion assay host for BCMV. We recently reported that bean cultivar 'Monroe' is an excellent local lesion host for quantitative assays of BCMV, and that some of the factors affecting lesion development have been defined (7). We now report on the distribution of BCMV in developing bean seed.

**Virus maintenance.**—The type strain of BCMV was routinely maintained in plants of the susceptible 'Mich-Cal Cranberry' (MCC) bean cultivar by periodic mechanical inoculation. Primary leaves of 10- to 15-day-old MCC plants were dusted with 0.05-mm (500-mesh) Carborundum, rub-inoculated with a pestle dipped into the virus preparation, and rinsed gently with distilled water. Plants were incubated at 24 C constant air temp under 16 hr of light daily at 16,140 lx (1,500 ft-c).

**Seed infection studies.**—BCMV distribution in reproductive tissues was examined in field-grown plants of the MCC variety. Both primary leaves of all plants were inoculated 2 wk after planting using the technique described above. Plants were examined for BCMV symptoms after an additional 3 wk, and those showing no typical symptoms were removed.

The effect of seed maturation on BCMV activity was examined by assaying seeds representing different stages of development (Fig. 1). Seeds were carefully removed from the pods (B, C, D, E) to prevent scratching and possible contamination from adjoining pod tissue. Seeds from three or four pods of each age group were combined, and two subsamples of three or four seeds each were selected at random. The seeds in one subsample were aseptically dissected into seedcoats, cotyledons, and embryos; these seed parts were then either surface-decontaminated by rinsing in running distilled water for 15 min or left untreated. The seeds in the second subsample remained intact.

The following inocula were prepared by trituration of bean tissue in the indicated volumes of buffer: (i) four unopened blossoms - 0.2 ml; (ii) four opened blossoms - 0.2 ml; (iii) three pods (A), including seeds - 0.2 ml; (iv) three pods (B, C) excluding seeds - 0.2 ml; (v) four seeds (B, C, D, E) - 0.2 ml; (vi) four seedcoats (C, D, E), nontreated - 0.2 ml; (vii) four seedcoats (C, D, E), surface-decontaminated - 0.2 ml; (viii) four pairs of cotyledons (C, D, E), untreated - 0.5 ml; (ix) four pairs of cotyledons (C, D, E), surface-decontaminated - 0.5 ml; (x) four embryos (C, D, E), nontreated - 0.2 ml; and (xi) four embryos (C, D, E), surface-decontaminated - 0.2 ml. Included in each infectivity assay was a triturated sample (1:4) of infected leaf tissue.

**Local lesion assays.**—Infectivity (local-lesion) assays were performed on 10-day-old plants of Monroe bean as described previously (7). Except for a few preliminary assays, all plants received 24 h of preinoculation darkness to enhance lesion formation. Within 1-2 h after inoculum preparation, inoculations were performed on Carborundum-dusted primary leaves, using a half-leaf method (4). Only a small amount of inoculum was available for most tissue preparations. Consequently, the pestle end was dipped directly into the inoculum and the inoculum was rubbed onto the leaf with the rough end of the pestle. Inoculated surfaces were immediately rinsed with a gentle stream of distilled water.

BCMV was distributed in various reproductive tissues of field-infected plants. Both unopened (6.0 lesions) and opened (5.9 lesions) blossoms, as well as small, juvenile pods (6.5 lesions) contained BCMV, indicating a relatively rapid invasion of these tissues after blossom initiation. BCMV was also detected in pod tissue during the subsequent period of rapid seed enlargement (B-2.0 lesions, C-9.9 lesions).

BCMV was found in both immature (B, C, D) and mature (E) seed developing on infected plants; infectivity was higher in the larger, more mature seeds (Table 1). The assay data from dissected seeds establishes that the virus is located primarily in cotyledons and embryos of infected seed. The presence of infectivity after surface-decontamination suggests that BCMV is internally-borne in these tissues. Low levels of infectivity associated with seedcoats were readily eliminated by surface-decontamination.

The distribution pattern of seed-transmitted BCMV in bean seed differs markedly from the distribution pattern of nonseed-transmitted southern bean mosaic virus (SBMV). Cheo (1) reported the presence of SBMV in both embryos and seedcoats of developing bean seeds.

TABLE 1. Infectivity (lesions per half leaf) of extracts from various parts of seeds from bean common mosaic virus-infected *Phaseolus vulgaris* 'Mich-Cal Cranberry'<sup>a</sup>

Pod appearance	Seedcoat <sup>b</sup>		Cotyledon <sup>b</sup>		Embryo <sup>b</sup>		Infected leaf
	U	S-d	U	S-d	U	S-d	
Green-Immature (C)	0.9	0.0	10.3	8.6	5.4	1.7	20.7
Striated-Slightly Mature (D)	1.1	0.0	16.3	21.6	10.9	9.6	21.5
Leathery-Dry and Mature (E)	0.0	0.0	15.3	13.7	13.0	6.5	21.5

<sup>a</sup>Average of four half-leaves per extract, and of six extracts per tissue sample.

<sup>b</sup>U = untreated tissue; S-d = tissue surface-decontaminated by 15-min rinse in running distilled water.

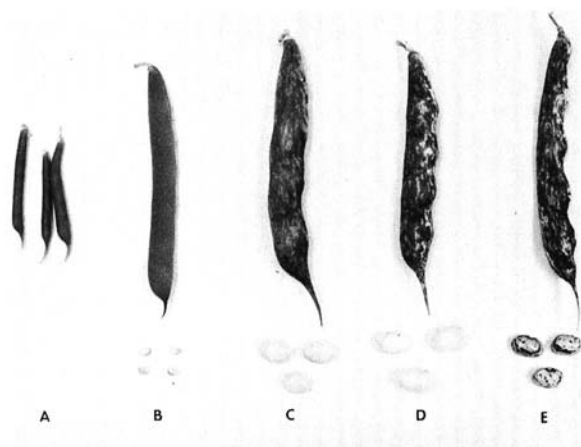


Fig. 1. Pods and seeds of 'Mich-Cal Cranberry' bean at various developmental stages before assay for bean common mosaic virus (BCMV). Seed samples C, D, and E were assayed both whole and after dissection into seedcoat, cotyledons, and embryo.

Although SBMV in the embryo was rapidly inhibited during seed maturation (drying), the virus in the seedcoat was unaffected (1). A more recent study indicates that SBMV is located primarily within the seedcoat tissue and that the infectivity associated with immature embryos is caused by external contamination from the seedcoat (5). The distribution patterns of cowpea chlorotic mottle virus in cowpea (3) and pea streak virus in pea (2), both

not seed-transmitted, are somewhat similar to that of SBMV, in that the viruses are specifically associated with the seedcoat; neither virus is associated with the embryo or cotyledons. Seed transmission is rare in such viruses.

However, seed transmission is common with BCMV. The present study suggests that successful seed transmission of BCMV is insured by the presence of infective virus within the embryo itself.

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