Distribution of Southern Bean Mosaic Virus in the Seed of Phaseolus vulgaris

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ABSTRACT

Southern bean mosaic virus was recovered from immature embryos and seedcoats harvested from SBMV-infected *Phaseolus vulgaris* 'Logan'. However, when the embryos and seedcoats were treated to remove surface contamination, infectivity was eliminated from the embryos, but not from the seedcoats. Virus infectivity was also recovered from decontaminated mature seedcoats. Phytopathology 62:387-389.

The seed transmission of southern bean mosaic virus (SBMV) in *Phaseolus vulgaris* L. was first reported in 1943 by Zaumeyer & Harter (5). These workers demonstrated the presence of infectious SBMV in mature seed that had been stored 7 months; 5% seed transmission of SBMV resulted when this seed was planted. Cheo (1) reported that infectious virus could be recovered from virtually all embryos and seedcoats of immature seeds of *P. vulgaris*, but from only seedcoats of mature ones, and he thus concluded that virus infectivity in the immature embryo was inhibited very rapidly upon dehydration of the seed at maturation. Unfortunately, in this study measures were not taken to decontaminate the dissected seed parts, a procedure other workers have found essential for reliable results (4). A re-examination of the distribution of SBMV infectivity in the seed of *P. vulgaris*, using decontamination procedures, was therefore desirable.

In the present study, SBMV, originally derived from AC.17 of the American Type Culture Collection, was used to establish infections in *P. vulgaris* 'Logan'. Inoculum was prepared by triturating frozen SBMV-infected leaf tissue (*P. vulgaris* 'Bountiful') in 0.01 M pH 7.1 potassium phosphate buffer (henceforth referred to as buffer). Plants were inoculated by the leaf-rubbing method at the primary leaf stage, and were maintained in a

controlled environment cabinet programmed to provide a photoperiod of 16 hr (2,200 ft-c) at 25 C, and a dark period of 8 hr at 20 C.

Infectivity assays were performed using the local lesion indicator host *P. vulgaris* 'Kentucky Wonder Wax'. Only primary leaves were used for such assays, and as inoculum could be applied to a single half-leaf, four samples could be inoculated to each plant. Assay plants were grown in a controlled environment cabinet programmed to provide a photoperiod of 16 hr (1,800 ft-c) at 25 C and a dark period of 8 hr at 20 C.

Prior to inoculation, assay plants were dusted lightly with 300-mesh Carborundum. Inocula were then applied to the four half-leaves, using the leaf-rub method with sterile gauze. Shortly after an inoculum had been applied, it was washed off with tap water. Local lesion counts were usually made 72 hr after inoculation.

When an infectivity assay was performed, one half-leaf of every plant was inoculated with a standard SBMV preparation. The standard preparation consisted of fresh sap from SBMV-infected leaf tissue diluted 200-fold in buffer; aliquots were stored in small tubes at −20 C and used throughout this investigation as required.

The distribution of SBMV infectivity in seeds harvested from SBMV-infected plants was determined by assaying dissected seed parts. Immature and mature seeds were dissected, using a sharp scalpel. Prior to dissection, mature seeds were soaked for 12 hr in distilled water to loosen the seedcoat from the embryo. Seed parts were decontaminated by washing them for 30 min in running tap water.

Immature and mature pods, each containing five seeds, were harvested from SBMV-infected plants. The seeds from each pod were dissected and inocula from the following treatments were prepared: (i) two embryos, decontaminated and triturated in 4 ml of buffer; (ii) two embryos, untreated and triturated in 4 ml of buffer; (iii) one seedcoat, decontaminated and triturated in 10 ml of buffer; and (iv) one seedcoat, untreated and triturated in 10 ml of buffer. Triturations were performed with mortars and pestles, and seedcoats were triturated with the aid of a small amount of sand (Standard Ottawa). These preparations were then inoculated, together with the SBMV standard (v) and a buffer control (vi) to assay plants. Two series of plants of eight replicates each were inoculated. In the first series of replicates, the two half-leaves of one leaf were inoculated with treatments i and ii, respectively. The two half-leaves of the opposite leaf were inoculated with preparations v and vi, respectively. In the second series of replicates, the two half-leaves of one leaf were inoculated with treatments iii and iv, respectively. The opposite leaf was inoculated as in the first series.

The results shown in Table 1 indicate that the infectivity associated with untreated embryos was eliminated by the decontamination procedure. This was in contrast to the results for seedcoats where no such effect was observed. Moreover, no infectivity was recovered from untreated mature embryos once maturation had taken place. Although infectivity could be recovered from mature seedcoats, there appeared to be a significant decline at maturation.

These results (Table 1) implied that infectious

TABLE 1. Infectivity (lesions per half-leaf) of extracts from embryos and seedcoats from seeds of southern bean mosaic virus (SBMV)-infected *Phaseolus vulgaris* 'Logan'[a]

Age of pod[b]	Embryos washed with tap water			Seedcoat washed with tap water			Embryos untreated			Seedcoat untreated			Buffer control			SBMV standard		
	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min
−12	0	1	0				29	66	6				0	0	0	41	63	18
				161	196	119				161	209	117	0	0	0	49	82	15
−6	0	0	0				7	14	1				0	0	0	24	66	7
				139	215	91				128	180	56	0	1	0	20	33	7
−3	0	0	0				6	14	2				0	0	0	14	38	5
				147	173	111				160	255	118	0	0	0	13	28	8
−3	0	0	0				11	25	3				0	0	0	18	34	5
				152	274	94				159	263	116	0	0	0	15	19	18
+3	0	0	0				0	0	0				0	0	0	18	34	5
				38	81	14				16	28	6	0	0	0	20	26	4
+4	0	0	0				0	0	0				0	0	0	28	71	5
				20	34	5				17	42	5	0	0	0	18	31	3
+8	0	0	0				0	0	0				0	0	0	17	31	7
				65	100	46				44	67	22	0	0	0	25	48	10
+9	0	0	0				0	0	0				0	0	0	16	34	4
				44	74	17				47	68	20	0	0	0	22	47	6

[a]Eight half-leaves/treatment.
[b]Expressed in approximate number of days from date of maturation.

virus should be recovered from the surface of the dissected embryo. When embryos were washed in a small amount of tap water or buffer, the wash was indeed found to be infectious. The probable source of contamination of immature embryos became apparent when these seeds were dissected. As the seedcoat was peeled off, thin layers of seedcoat tissue remained attached to the embryo. When the embryo was washed in a small amount of water, these membranous layers came off the embryo. Extracts from the untreated mature embryo were not infectious, but here the situation was quite different. The dead, mature seedcoat was loosened from the embryo by soaking. The result was that the seedcoat, which was then compact and leathery, came away completely from the embryo. The actual removal of the seedcoat was easy, and no pieces of tissue were left behind. When the mature embryo was washed in a small amount of water, there were no extraneous pieces of tissue visible.

Southern bean mosaic virus has been reported to be transmitted in mature seed of *P. vulgaris* (1, 2, 5) and *Vigna sinensis* (3). Our results support the previous finding (1) that in the mature seed of *P. vulgaris*, infectious SBMV is confined to the seedcoat. If this is indeed so, and SBMV is not present in the mature embryo in some undetectable form, then the probably mechanism of seed transmission in *P. vulgaris* (and possibly in other hosts of SBMV) is by infection of the embryo at germination with virus from the seedcoat. A mechanism similar to this has been postulated (4) for the seed transmission of tobacco mosaic virus in *Lycopersicon esculentum*.

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