Seed Transmission of a Brazilian Isolate of Soybean Mosaic Virus

M. D. M. Porto and D. J. Hagedorn

Research Assistant and Professor, respectively, Department of Plant Pathology, University of Wisconsin, Madison 53706. Present address of senior author: Faculdade de Agronomia, UFRGS – Porto Alegre, RS, Brazil.

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, with funds provided by USAID/Wisconsin Contract LA-147.

Accepted for publication 3 February 1975.

ABSTRACT

A Brazilian isolate of the soybean mosaic virus (SMV) was consistently found in the embryos and cotyledons of mature soybean seeds. Seeds harvested from virus-infected plants of 12 different soybean cultivars were tested for transmission of the virus, mottling of the testa, and germination. Extent of mottling of the testa and seed transmission of the virus were dependent on the soybean cultivar. Neither the mottling nor presence of the virus had any effect on germination. Tests with mottled seeds produced by noninoculated soybean plants showed that mottling was not necessarily induced by virus infection.

Phytopathology 65:713-716

Additional key words: *Glycine max*, embryos, cotyledons, soybean seed mottling.

Soybean mosaic virus (SMV) was first reported to be seed-transmitted in 1921 by Gardner and Kendrick (3). Three years later, they reported that the disease could overwinter in the seeds lying in the field. They also observed that mottled seeds were produced by both healthy and diseased plants, and that infected seedlings were obtained from nonmottled as well as from mottled seeds (5).

The mottling of soybean seeds was attributed to the effects of hereditary and environmental factors (7). Association between mottling of the testa and virus infection was first established in 1957 (6). In some cultivars, the percentage of mottled seeds produced by virus-inoculated soybean plants was two or three times higher than that from noninoculated plants (8). It has been suggested recently that mottled seeds are produced only by virus-infected soybean plants (10), and that mottling on seeds from noninoculated plants reflects the natural transmission of the virus (9). However, evidence to support these hypotheses has not been presented.

Association between genotype of the SMV-infected plant and extent of mottling has also been demonstrated. Wilcox and Laviolette (11) observed that SMV-infected soybean plants, which had the genotype *ITrw* (yellow hilum), produced 78% of brown-mottled seeds; those with *ItRw* genotype (yellow hilum) produced 38% of buff-mottled seeds, and those with *iitrW* (buff hilum) produced only 4% of buff-mottled seeds.

The amount of seed transmission has been affected by the time of inoculation; i.e., the earlier the inoculation, the larger the percentage of infected seeds (9). This percentage, however, did not materially affect the germination of the seeds. Different soybean cultivars have shown different rates of seed transmission (4).

The present study was undertaken to determine whether a Brazilian isolate of SMV would induce the same phenomena of seed transmission as those reported for other isolates. Emphasis was placed on experiments to (i) determine in which part(s) of the soybean seeds the virus particles were carried, and (ii) determine whether seed mottling was completely dependent upon virus infection.

MATERIALS AND METHODS.—*Location of soybean mosaic virus in the soybean seeds.*—All seeds used in this research were obtained from SMV-infected soybean plants, cultivar Bansei, which had been

inoculated with a Brazilian isolate of the virus and grown in an air-conditioned greenhouse at 22-24 C. The SMV isolate was obtained in 1970 near Veranopolis, Rio Grande do Sol.

Mottled seeds were soaked in distilled water for 5 hours and carefully dissected into their components: testas, embryos, and cotyledons. The cotyledonary regions adjacent to the embryo were removed and discarded. Nonmottled seeds were also dissected and tested.

The three seed components of 10 mottled seeds were treated in three ways. They were (i) surface decontaminated, (ii) soaked in SMV, then surface decontaminated and (iii) used without decontamination. The second treatment was to insure the effectiveness of the decontamination procedure which consisted of soaking seed components for 30 seconds in a 6.4% solution of $NaHClO_3$ followed by thorough rinsing with distilled water. Nonmottled seed components were studied only after decontamination.

All seed parts, prepared as described, were ground in a mortar and suspended (1:5, w/v) (grams:ml) in distilled water. The inoculation was performed by rubbing with a cheesecloth pad soaked in the inoculum the two primary leaves of soybean plants, cultivar Bansei. The water in which mottled and nonmottled seeds had been soaked was also inoculated onto primary leaves of the test plants.

To eliminate the possibility that virus movement occurred via contamination during these soaking periods, two more experiments were performed in which the soaking period was omitted. Nearly mature soybean seeds, which could be dissected without a previous soaking period, were used in test number 2. Procedures for decontamination of the seed parts and inoculation onto the test plants were the same as those described for the previous test.

In the third test, mature seeds were studied for presence of virus. A comparison was made between the three seed parts which were submitted to a 5-hour soaking period and those placed on a wet blotter. The testas from the seeds placed on the blotter could be removed in 2 days. This test consisted of 12 treatments: six treatments for the presoaked seeds = decontaminated and nondecontaminated testas, cotyledons and embryos; and a similar set of six treatments for the seeds which had been placed on the wet blotter.

The relationship between seed mottling, SMV-transmission, and seed germination.—Using seeds from SMV-infected plants, the percentage of mottling, seed-transmission, and germination was determined in seed from 12 soybean cultivars. Two hundred seeds of each cultivar were planted in a steamed mixture of soil:sand:peat (2:1:1). The plants were grown for 2 months, during which repeated attempts were made to recover the virus from symptomless plants by inoculation onto leaves of the soybean cultivar Bansei. The experiment was repeated twice.

Experiments were undertaken to detect soybean mosaic virus in mottled seeds known to have been produced by noninoculated soybean plants. Large-scale tests were performed twice, using Bansei seeds obtained from two different sources. Five hundred seeds from a heavily mottled seed lot, obtained from Urbana, Illinois, were planted in white sand and maintained in a growth chamber for 1 month. The environmental conditions in the growth chamber were: a 14-hour photoperiod with 19,368-21,520 lx light intensity; temperature in the light was 25 C and in the dark 18 C. The second lot of mottled seeds was obtained from field plots in Madison, Wisconsin. Seeds were harvested at random from 40 symptomless noninoculated soybean plants. Seeds were also collected at random from SMV-inoculated plants. One thousand mottled seeds from the noninoculated, and the same amount from the SMV-inoculated plants, were planted in vermiculite and kept for 1 month under greenhouse conditions of 24 C. The seedlings from the two seed lots were kept in two different greenhouses to prevent any possibility of natural transmission of the virus.

TABLE 1. Transmission of soybean mosaic virus in the different parts of soybean seeds

Test	Treatment	Seed part		
		Testa	Cotyledon	Embryo
1	Mature seeds - soaked in distilled water:			
	decontaminated	–[a]	+[b]	+
	nondecontaminated	+	+	+
2	Near-mature seeds - not soaked:			
	decontaminated	–	+	+
	nondecontaminated	–	+	+
3	Mature seeds - soaked in distilled water:			
	decontaminated	–	+	+
	nondecontaminated	+	+	+
	Mature seeds - placed on wet blotter:			
	decontaminated	–	+	+
	nondecontaminated	–	+	+

[a]The – indicates that infection was not detected in the test plants.
[b]The + indicates that test plants became infected with soybean mosaic virus.

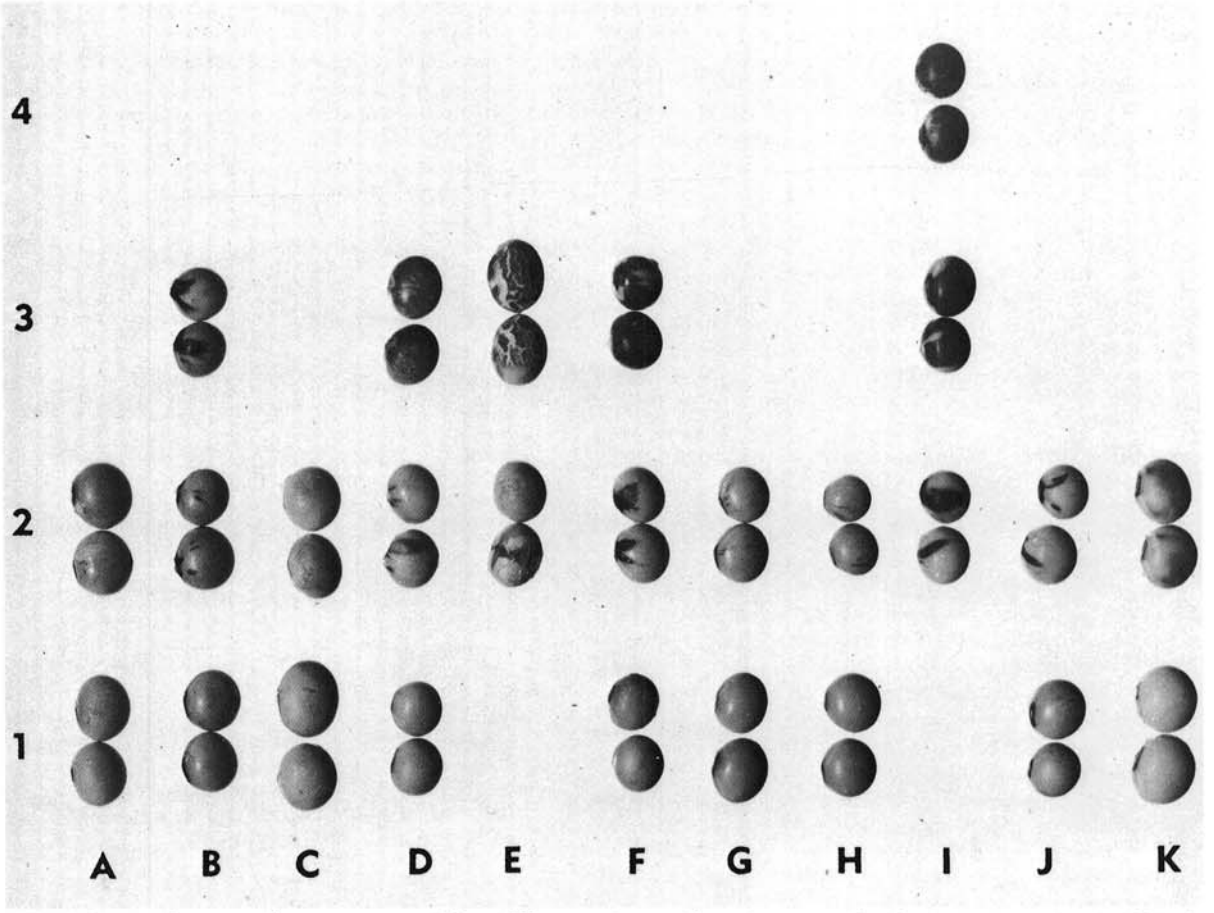

Fig. 1. Degree of testa mottling present on seeds from different soybean cultivars. 1 = no mottling; 2 = dark color on less than 25% of the testa surface; 3 = dark color on more than 25% of the testa surface; 4 = testa completely dark. Soybean cultivars: A – Bienville; B – Bragg; C – Halle 7; D – Hardee; E – Harosoy 63; F – Hill; H – Jackson; I – Santa Rosa; J – Industrial; K – Majos.

RESULTS.—*Location of soybean mosaic virus in the soybean seeds.*—The solution containing 6.4% $NaHClO_3$ was a good decontaminant of SMV. Eight test plants inoculated with the crude juice from SMV-infected plants were readily infected, but those inoculated with ground testas, which had been immersed in the crude juice prior to decontamination, did not develop symptoms of virus infection.

Soybean mosaic virus was consistently transmitted by the cotyledons and embryos of mature soybean seeds (Table 1). Transmission of the virus by the testas seemed to be merely a product of contamination, because any transmission through this part of the seed could be easily prevented by treating it with $NaHClO_3$.

The absence of virus in testas from seeds which were not soaked suggested that the contamination might have occurred during the soaking period. Additional evidence for this hypothesis is that the virus was found in the water in which the seeds were soaked.

The relationship between seed mottling, SMV-transmission, and seed germination.—Neither the mottling of the testa nor the presence of SMV particles had any effect on seed germination (Table 2).

The 12 cultivars tested showed a wide variation in testa mottling and transmission of soybean mosaic virus. Only cultivars Harosoy 63 and Santa Rosa showed 100% mottled seeds. These two cultivars also gave the highest levels of seed transmission of the virus.

TABLE 2. The relationship between seed mottling, virus seed transmission, and seed germination in soybean mosaic virus-infected plants from 12 soybean cultivars

Soybean cultivars	Virus transmission (%)	Mottled seeds (%)	Germination (%)
Bienville	0[a]	91	94
Bragg	9	79	97
Corsoy	15	73	81
Halle 7	0	24	88
Hardee	23	82	98
Harosoy 63	64	100	89
Hill	7	90	95
Hood	0	10	100
Industrial	3	80	100
Jackson	0	15	96
Majos	0	8	98
Santa Rosa	40	100	89

[a]The average of two experiments.

The soybean cultivar Bienville did not transmit the virus through the seeds, but it showed a high percentage of mottling (Table 2). In this cultivar, the extent of mottling was never more than 25% of the testa (Fig. 1) and the mottling was also common on seeds from noninoculated plants. Production of mottled seeds by supposedly virus-free plants was also observed in the cultivars Hardee, Hood, Industrial, and Majos. All these plants had grown under conditions where the possibilities of natural spread were greatly reduced because of repeated insect control treatments.

The color of the dark patches on the mottled testas followed the same pattern described by Wilcox and Laviolette (11).

Soybean mosaic virus was not found associated with mottled seeds produced by noninoculated soybean plants. Transmission of the virus through the seeds of inoculated soybean plants, cultivar Bansei, ranged from 14 to 17%.

The seedlings obtained from SMV-infected seeds began to show symptoms of virus infection about 10 days after germination. The primary leaves of the diseased plants were misshapen and their edges were curved downward. Typical symptoms of SMV infection were shown on the first and subsequent trifoliate leaves. No vein-clearing stage was observed.

DISCUSSION.—The location of the virus particles in the cotyledons and embryos of mature seeds coincides with that reported for tobacco ringspot virus (1). It has been reported that soybean mosaic virus was found associated with the testas of immature seeds (4). We were unable to detect, except in cases where contamination was evident, the presence of infectious virus in the testas of mature seeds. Such results may indicate that virus particles present in the testa were inactivated during the process of maturation of the seeds.

The use of virus-free seeds has been suggested as a measure for controlling soybean mosaic (2). Under such a plan, one way to obtain virus-free seeds would be by elimination of mottled seeds, or by establishing a limit for mottled seeds, over which the seed lot could not be used for planting. This recommendation was probably based on the assumption that mottled seeds are produced only by infected plants. Such a method has little or no merit because there was only an inconsistent relationship between mottling of the testa and transmission of soybean mosaic virus. The present research indicates that some soybean cultivars do produce virus-free mottled seeds. Therefore, to recommend elimination of mottled seeds would be to overestimate the usefulness of this characteristic. Such recommendation could be detrimental to seed producers because it would only minimize the transmission of soybean mosaic virus through the seeds and not completely eliminate this source of inoculum.

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