Similarities in Seed and Aphid Transmission Among *Soybean mosaic virus* Isolates

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ABSTRACT

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Soybean mosaic virus (SMV) is an aphid- and seed-transmitted virus that infects soybean (*Glycine max*) plants and causes significant yield losses. Seed-borne infections are the primary sources of inoculum for SMV infections. The strain specificity of SMV transmission through seed and SMV-induced seed-coat mottling were investigated in field experiments. Six soybean plant introductions (PIs) were inoculated with eight SMV strains and isolates. Transmission of SMV through seed ranged from 0 to 43%, and isolate-by-soybean line interactions occurred in both transmission rates and percentages of mottled seeds. For example, SMV 746 was transmitted through 43% of seed in PI 229324, but was not transmitted through seed of PIs 68522, 68671, or 86449. In contrast, SMV 413 was transmitted through seed from all PIs. SMVs that were transmitted poorly by the Asian soybean aphid, *Aphis glycines,* also were transmitted poorly through seed. No predicted amino acid sequences within the helper-component protease or coat protein coding regions differentiated the two groups of SMV strains. The loss of aphid and seed transmissibility by repeated mechanical transmission suggests that constant selection pressure is needed to maintain the regions of the SMV genome controlling the two phenotypes from genetic drift and loss of function.

Additional keywords: posttranscriptional gene silencing

Soybean mosaic virus (SMV) is an aphid- and seed-transmitted member of the *Potyviridae* that infects soybean (*Glycine max* L.) plants and causes significant losses in the amount and quality of seeds harvested (18,20). Since SMV rarely infects alternative host species, seed-borne infections are the primary sources of inoculum for SMV infections (18). Controlling seed-borne SMV infections has become more important with the discovery of *Aphis glycines* Matsumara in North America, which can efficiently transmit SMV among soybean plants (10,13,19). SMV isolates can be divided into strains (G1 through G7) based on the symptoms they produce on a differential set of soybean lines (9,36). In addition to differing in symptom severity, SMV strains G1 through G7 also differ in the efficiency with which they are transmitted through seeds of different soybean lines (7).

As with other members of the *Potyviridae*, the efficiency with which SMV is transmitted through seed is dependent upon the strain of virus analyzed and the genotype of the host. While differences in seed transmission of *Pea seed borne mosaic virus* (PSbMV) were associated with the inability of poorly seed-transmitted virus isolates to invade embryos (42), Bowers and Goodman (6) reported that cultivar-specific differences in seed transmission of a severe isolate of SMV were related to differences in the abilities of SMV strains to remain infectious within maturing embryos. Seed-transmissible strains of PSbMV were shown to enter developing embryos through a transient symplastic pathway that connects the base of the suspensor to the developing embryo (37). Isolates of PSbMV not transmitted through seed infected the testa, but were unable to invade and infect developing embryos. In contrast, infectious SMV was found in both the testa and embryo of immature seeds of a soybean cultivar with very low seed transmission, but only in the testa after seeds had desiccated (6).

Johansen et al. (25) showed that regions of the PSbMV genome that encode the helper component/protease (HC-Pro) and coat protein (CP) contained determinants for seed transmission. HC-Pro is a multifunctional protein that, in addition to its role in seed transmission, facilitates aphid transmission (33) and long-distance movement (12), binds RNA (28), and is a potent suppressor of posttranscriptional gene silencing (PTGS) (2). Much like HC-Pro, the γb protein of *Barley stripe mosaic virus* (BSMV) has been shown to be involved in both seed transmission and suppression of PTGS (14,44). Similarly, the 12K protein of *Pea early browning virus* is a determinant of seed transmission (41), and the corresponding protein of *Tobacco rattle virus* is a suppressor of PTGS (35). These findings suggest that specific movement and/or protection of viral RNA from PTGS-mediated degradation are involved in transmission of viruses through seed.

Bowers and Goodman (7) also reported SMV strain-by-soybean line interactions in seed-coat mottling. In cultivated soybean, the distribution of anthocyanin and proanthocyanidin pigments in seed coats is controlled by four alleles at the *I* locus (4). Alleles that suppress the accumulation of pigments in seed coats contain inverted repeats of the chalcone synthase (CHS) gene cluster (11,40). The structure of the *I* locus leads to PTGS of CHS mRNAs that results in yellow-colored soybean seeds. SMV infections, presumably through the action of HC-Pro, induce seed-coat mottling by partially suppressing silencing of the CHS mRNAs (38). The strain-by-line interactions in seed transmission and seed-coat mottling suggest that there are very specific interactions of virus and host components in movement of SMV into soybean embryos and/or survival of SMV in maturing seeds and suppression of silencing.

The genetics of seed transmission also have been examined. With hosts of BSMV, resistance to seed transmission is controlled by a single recessive gene (8). In contrast, seed transmission of PSbMV and *Alfalfa mosaic virus* is controlled by multiple genes in a quantitative manner (32,43). The genetics of resistance to seed transmission of SMV have not been characterized (22).

In this study, we compared seed transmission of four laboratory strains of SMV

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(G2, G5, G7, and G7F) that had been maintained by mechanical inoculation and four field SMV isolates (413, 746, 1083, and 88799) that had been maintained by aphid or seed transmission, with the goal of establishing a system to investigate viral and host determinants of strain-specific transmission of SMV through seed. Because of the roles of HC-Pro and virus stability in transmission of potyviruses through seed, the predicted amino acid sequences of the HC-Pro and CP coding regions were determined for each virus isolate and compared to their seed and aphid transmission phenotypes.

MATERIALS AND METHODS

Plant material and virus isolates. Seeds of plant introductions (PIs) PI 68522, PI 68671, PI 84657, PI 86449, PI 88799, and PI 229324 were obtained from the USDA Soybean Germplasm Collection, Urbana, IL. Prior to use in seed transmission studies, each PI was grown in an insect-free greenhouse, and seeds were collected from plants that were negative for SMV infection by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using antibodies from Agdia (Elkhart, IN). SMV strains G2 and G7 were provided by J. Hill (Iowa State University, Ames); SMV G5 was obtained from American Type Culture Collection (Manassas, VA); and G7F was recovered from an original culture of Cho and Goodman (9). SMV isolates 413, 746, and 1083 were collected from field plots on the Crop Sciences Research and Education Center in Urbana, IL (13). SMV isolate 88799 was obtained from infected seeds of PI 88799. SMV strains G2, G5, G7, and G7F were maintained by mechanical inoculation. SMV isolates 413, 746, and 1083 were maintained through transmission by *A. glycines* on cultivar Williams 82. SMV isolate 88799 was maintained by seed transmission on PI 88799. Virus extracts for mechanical inoculations were prepared from infected soybean plants maintained in the greenhouse by grinding infected leaves with sterilized mortars and pestles in chilled 25 mM potassium phosphate buffer, pH 7.1, 10 mM Na_2SO_3. Unifoliate leaves were dusted with Carborundum (320 grit, Fisher, Fairlawn, NJ) and inoculated by rubbing with pestles dipped in inoculum.

Seed and aphid transmission. Field experiments were conducted to compare the rates at which isolates of SMV were transmitted through seed. The six soybean PIs were inoculated separately at the unifoliate growth stage with eight SMV strains and isolates (G2, G5, G7, G7F, 413, 746, 1083, and 88799). All infections were confirmed by DAS-ELISA. Plants were grown to maturity and seeds were harvested and stored at 4°C. Seed transmission rates were determined by planting up to 200 seeds from infected plants in 96-well polystyrene trays containing soilless mix (Sunshine Mix LC1, Sun Gro Horticulture Inc., Bellevue, WA). SMV infections were detected using a tissue blot assay (27). For virus–host combinations that produced less than 200 seeds, all available seeds were analyzed. Samples with ambiguous results from tissue blots were retested by ELISA.

Field experiments were conducted during 2003 and 2004 in field cages 3 × 3 m and 1.5 × 3 m on the Crop Sciences Research and Education Center in Urbana, IL. Cages were covered with 32-mesh screen to exclude insect vectors. Single row plots, 0.5 m in length and 0.6 m between rows, were hand sown to obtain six plants per row (one plant of each PI line). Single row plots were inoculated with an individual virus isolate. Plots were arranged in a randomized incomplete block design with three replications. Analysis of variance of seed transmission and mottling data were performed using SAS (version 9.13; SAS Institute, Cary, NC).

Colonies of *A. glycines* were maintained in controlled environment chambers on cultivar Williams 82, which was also used for the aphid transmission assays. Alate aphids were collected and starved for at least 30 min and given 2-min access feeds on leaf tissue infected with SMV 88799. Five aphids were transferred to each of 10 soybean seedlings and allowed to feed for at least 24 h before being transferred to a second environment chamber for 48 h in which a Hot Shot No-Pest Strip (Chemsico, St. Louis, MO) had been mounted. Subsequently, inoculated plants were maintained in a greenhouse for 4 weeks and assayed by tissue blot assay. Aphid transmission phenotypes for SMVs G2, G5, G7, G7F, 413, 746, and 1083 were determined previously (13).

Sequence analysis. The HC-Pro and CP sequences of SMV G2, G5, G7, G7F, 413, 746, and 1083 were determined previously (13). HC-Pro and CP nucleotide sequences of SMV 88799 were determined by direct sequencing of polymerase chain reaction (PCR) products (13). Sequences were aligned using ClustalX (23) and edited with GeneDoc (30).

RESULTS

Seed transmission and mottling. In both trials, SMV strains were differentially transmitted through seed (Table 1). Transmission of SMV through seed ranged from 0 to 43% depending on the soybean line and virus strain analyzed (Table 1). In addition, significant ($P < 0.001$) SMV isolate-by-soybean line interactions were seen in germination and seed-coat mottling (Tables 1 and 2). SMV isolates 746 and 88799 showed the largest differences in transmission rates among soybean lines. More than 20% of the seedlings germi-

Table 1. Percent transmission of *Soybean mosaic virus* (SMV) isolates through seed and percent germination of seeds

SMV strain	Trial[w]	Percent germinated seedlings infected with SMV											
		PI 68522		PI 68671		PI 84657		PI 86449		PI 88799		PI 229324	
G2	1	0[x]	(86)[y]	0	(70)	1	(10)	0	(70)	1	(69)	0	(48)
	2	0	(74)	0	(48)	1	(97)	0	(90)	4	(74)	0	(80)
G5	1	0	(92)	0	(70)	4	(44)	0	(92)	2	(98)	19	(90)
	2	0	(85)	1	(88)	1	(100)	0	(93)	2	(96)	13	(72)
G7	1	1	(54)	0	(38)	0	(8)	0	(58)	0	(24)	8	(59)
	2	0	(80)	0	(33)	1	(86)	1	(91)	5	(98)	0	(100)
G7F	1	0	(44)	0	(37)	0	(12)	0	(48)	0	(34)	0	(71)
	2	0	(80)	0	(39)	0	(85)	0	(100)	0	(67)	0	(43)
413	1	8	(71)	32	(78)	19	(20)	6	(78)	17	(60)	31	(77)
	2	13	(81)	24	(75)	29	(98)	5	(66)	32	(84)	24	(96)
746	1	0	(79)	6	(72)	0	(62)	0	(100)	28	(80)	43	(90)
	2	0	(96)	0	(92)	0	(96)	0	(95)	37	(100)	32	(100)
1083	1	10	(30)	20	(66)	13	(74)	0	(92)	16	(71)	33	(83)
	2	5	(64)	0	(50)	0	(73)	5	(78)	12	(73)	NS[z]	(NS)
88799	1	0	(93)	0	(65)	7	(82)	0	(97)	29	(94)	40	(92)
	2	0	(89)	0	(93)	0	(96)	0	(68)	20	(92)	22	(99)

[w] Trial 1 was conducted during 2003; trial 2 was conducted during 2004. When available, 200 seeds were planted for each line.
[x] Percent germinated seedlings infected with SMV.
[y] Percent seeds germinated.
[z] No seeds produced.

Table 2. Percent mottled seeds from *Soybean mosaic virus* (SMV)-infected field-grown plants

SMV strain	Trial[x]	Percent seeds showing seed-coat mottling					
		PI 68522	PI 68671	PI 84657	PI 86449	PI 88799	PI 229324
G2	1	32[y]	69	93	38	98	100
	2	43	62	61	41	96	100
G5	1	4	6	4	0	81	82
	2	0	0	0	0	77	74
G7	1	29	59	86	19	100	87
	2	9	30	84	14	95	100
G7F	1	24	62	94	31	98	100
	2	7	71	96	11	100	100
413	1	75	70	62	60	98	100
	2	63	39	94	77	96	100
746	1	0	0	0	0	78	79
	2	0	0	0	0	85	80
1083	1	62	60	67	32	100	93
	2	84	61	93	81	100	NS[z]
88799	1	0	0	0	2	86	76
	2	0	0	0	0	89	77

[x] Trial 1 was conducted during 2003; trial 2 was conducted during 2004.
[y] Percent mottled seeds from samples of 200 seeds, when available.
[z] No seeds produced.

Table 3. Transmission of *Soybean mosaic virus* (SMV) through seed and seed-coat mottling of six soybean plant introductions

	Seed transmission	Seed mottling
PI 68522	2.3 ab[y]	27.0 a[z]
PI 68671	5.1 b	36.8 b
PI 84657	4.7 b	52.1 c
PI 86449	1.2 a	25.4 a
PI 88799	12.8 c	92.3 d
PI 229324	17.6 d	89.9 d

[y] Values within a column with the same letters are not significantly different ($P < 0.05$). Least significant difference (LSD) = 3.2.
[z] LSD = 7.2.

nated from field-grown plants of PIs 88799 and 229324 infected with SMVs 746 and 88799 were infected with SMV. But none of the seedlings from similarly field-grown and infected plants of PIs 68522 and 68671 were infected with SMV 746 or 88799. In contrast, SMV 413 was transmitted through seed from all PIs at rates ranging from 5 to 32%. No seed transmission was detected of SMV G7F from either year in the field trials. Data from the 2 years did not differ significantly ($P > 0.1$). The rates of seed transmission (correlation coefficient = 0.85) were highly correlated between years of the field trials, as were percentages of mottled seeds (correlation coefficient = 0.93).

As with seed transmission, significant ($P < 0.001$) SMV isolate-by-soybean line interactions were seen in percentage of seed-coat mottling (Table 2). Soybean lines were separated into four groups based on their mottling responses to different SMV strains (Table 3). At least 74% of the seeds of PI 88799 and PI 229324 were mottled from plants infected with any one of the eight SMV isolates. In contrast, when the results from the 2 years were averaged, 3% or less of the seeds of PIs 68522, 68671, 84657, and 86449 were mottled when plants were infected with SMV G5, 746, and 88799. When the same PIs were infected with SMV strains G2, G7, G7F, 413, and 1083, the average percentage of mottled seeds over the 2 years ranged from 15 to 95%. Soybean lines were ranked similarly based on their percentages of seed transmission and seed-coat mottling. PIs 68522 and 86449 had among the lowest mean percentages of seed transmission and seed-coat mottling. PIs 88799 and 229324 had the highest mean percentages of seed transmission and seed-coat mottling (Table 3).

Similar patterns were seen in aphid and seed transmission (Table 4). As a group, SMV isolates that had been maintained by mechanical inoculation were transmitted by aphids and through seed at significantly lower rates (0% aphid transmission and 1% seed transmission) than SMV isolates that had been maintained by *A. glycines* or seed transmission (50% aphid transmission and 13% seed transmission).

Sequence analysis. The HC-Pro coding regions of all of the highly seed transmissible SMV strains, except SMV 88799, shared two amino acids that the poorly transmitted strains lacked, an N at position 65 and a K at position 100 compared with H or Q and R, respectively, in the poorly transmitted strains (Table 4). The predicted CP amino acid sequences of all highly seed transmissible strains (413, 746, 1083, and 88799) had the DAG amino acid triplet near their amino termini, while the CP sequences of G5, G7, and G7F contained DAD, GAD, and GAD triplets, respectively. Even though SMVs G2 and 88799 differed in their transmission through seed in field experiments, their HC-Pro and CP predicted amino acid sequences were identical except for an N to S substitution at position 449 in HC-Pro.

DISCUSSION

In this study, we compared the abilities of eight SMV strains to induce seed-coat mottling and to be transmitted through seed and by aphids. As described by Pacumbaba (31), seed coat mottling was not a good indication of seed transmissibility of SMV isolates. Even so, when soybean lines were inoculated with seed-transmissible SMV isolates, seed coat mottling and transmissibility were correlated. Differential transmission of SMV through seed by soybean lines and strain-by-line variation in seed transmission and seed coat mottling have been reported previously (5–7,17). Much of the previous analyses were conducted using the Illinois severe isolate of SMV (SMV-Il-S), which was later reclassified as SMV G2 (9). In experiments conducted by Bowers and Goodman (7), SMVs G1 through G7 all were transmitted efficiently through seed for a set of soybean lines. In the present study, however, SMVs G2, G5, G7, and G7F were transmitted poorly through seed of all soybean lines analyzed. For example, SMV-Il-S was transmitted through 29 and 34% of seed of PIs 84657 and 229324, respectively (5); but in this study, SMV G2 was transmitted through seed at rates of 1 and 0% in the same PIs. These differences in seed transmissibility probably result from the repeated mechanical transmission of the laboratory strains that has occurred in the nearly 30 years since the original experiments were conducted.

Repeated serial transmission of potyviruses has been shown to lead to loss of aphid transmissibility (15,39), which has been associated with amino acid sequence changes in the HC-Pro and CP coding regions (29). Mutations in three amino acids, DAG, near the amino terminus of the CP have been shown to result in loss of transmission by aphids for multiple potyviruses (3,21). In this study, most poorly seed- and aphid-transmitted SMV isolates had mutations in the DAG motif. However, some potyviruses, e.g., isolates of PSbMV, do not have DAG triplets and are still transmitted efficiently by aphids and through seed (26). While HC-Pro and CP have been implicated in both aphid and seed transmission (26), different regions of the proteins may be involved in the two modes of transmission. It is also possible that mechanical transmission of SMV exerted a selection for virus structures that were less compatible with aphid and seed transmission.

The association of seed and aphid transmissibility suggested that sequences required for aphid and seed transmission need constant selection to avoid genetic drift and loss of function. Recently, Ali et al. (1) showed that aphid transmission of *Cucumber mosaic virus* represented a bottleneck that reduced the number of mutations in transmitted viruses. Similarly, transmission of SMV through seed resulted in the selection of viruses that often

Table 4. Predicted helper component/protease (HC-Pro) and coat protein (CP) amino acid sequences, percent seed-coat mottling and seed and aphid transmission phenotypes of *Soybean mosaic virus* (SMV) strains

	HC/Pro																															CP							Percent transmission		
SMV strain	3	6	22	30	48	55	65	83	84	88	89	91	92	96	100	104	116	170	227	237	257	271	307	342	374	413	449	10	12	18	22	27	101	264	% Mottling	Seed	Aphid
G2	-[x]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	-	P	69.5 cd[y]	0.6 a[z]	0/30
G5	-	-	N	-	-	-	Q	-	T	-	-	-	-	K	-	-	-	-	I	-	N	P	-	-	Q	K	-	-	D	N	-	-	-	-	27.3 a	3.5 a	0/29
G7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	D	-	-	-	V	-	59.4 b	1.3 a	0/30
G7F	-	D	-	T	-	-	-	-	-	-	-	-	-	-	-	I	-	T	-	-	-	-	R	-	-	-	-	G	D	-	-	-	-	P	66.1 bc	0.0 a	0/29
413	T	-	-	-	V	-	N	R	-	N	I	K	V	K	K	-	M	-	I	-	N	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	77.8 d	19.9 c	20/30
746	-	V	-	-	-	-	N		T	-	-	-	-	-	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26.9 a	12.0 b	9/30
1083	T	-	-	-	V	-	N	R	-	N	I	K	V	K	K	-	M	-	I	T	-	-	-	Y	-	-	-	-	-	-	-	-	A	-	-	76.4 d	10.3 b	23/30
88799	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	27.5 a	9.9 b	8/30
consensus	N	A	H	I	I	S	H	G	A	T	F	E	I	R	R	T	L	A	V	I	D	T	K	F	R	Q	S	D	G	S	S	T	D	Q			

[x] Amino acids are same as consensus.
[y] Values within a column with the same letters are not significantly different ($P < 0.05$). Least significant difference (LSD) = 8.4.
[z] LSD = 3.7.

had different virulence phenotypes than the virus used for inoculation (16).

The findings of Johansen et al. (25) that CP and HC-Pro coding regions of the PSbMV genome were required for efficient transmission through seed of *Pisum sativum* are consistent with the association between aphid and seed transmissibility observed here. For a virus to be transmitted through seed, it must infect embryos and survive seed desiccation (24). Brown and Goodman (6,34) showed that both seed-transmissible and nontransmissible strains of SMV invaded soybean embryos, but only SMVs that remained infectious were transmitted through seed. The involvement of CP may be related to differential stabilities of virions of different isolates. Variation in HC-Pro amino acid sequence may alter the protein's ability to bind virions, aphid stylets, or RNA, or its ability to suppress PTGS. Since seed transmission is an important source of SMV infections (18), viruses that are efficiently transmitted both vertically and horizontally would, undoubtedly, be more readily dispersed within and among soybean fields than viruses lacking one of these modes of transmission. Additional studies will be required to identify the SMV and soybean genes involved in seed transmission.

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