Seed Transmission of Soybean Mosaic Virus in Mottled and Nonmottled Soybean Seeds

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ABSTRACT

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Differences in the rates of soybean mosaic virus seed transmission among cultivars and lines of soybean, *Glycine max* (L.) Merr., were determined by double antibody sandwich enzyme-linked immunosorbent assay of seedlings. The presence of soybean mosaic virus in 5- to 6-wk-old soybean seedlings varied significantly among 11 cultivars and lines. The virus was detected in plants grown from mottled and nonmottled seeds, but the difference was not significant. Virus-infected plants grown from mottled seeds produced 71.5% mottled and 28.5% nonmottled seeds. Virus-free plants grown from mottled seeds produced 65.2% mottled and 34.8% nonmottled seeds. Virus-infected plants grown from nonmottled seeds produced 81.9% mottled and 18.1% nonmottled seeds. Virus-free plants grown from nonmottled seeds produced 70.8% mottled and 29.2% nonmottled seeds. Mottling of soybean seeds was not indicative of the presence of soybean mosaic virus in plants grown from these seeds or of the incidence of mottled seed produced by these plants.

Soybean mosaic, caused by soybean mosaic virus (SMV) of the potyvirus group, is a common disease of soybean, *Glycine max* (L.) Merr., and a potentially serious threat to the soybean industry in some areas of the United States (6). Yield losses (21–24), reduced seed quality (15, 22,23), decreased oil content (9), and decreased nodulation (27) have resulted from this disease.

The virus is transmitted nonpersistently by aphids (1,7,11,16) and through seeds (3,6,10,13,14,16,23). Seed coat mottling produced on some SMV-infected plants results when the seed coat of yellow-seeded soybean varieties or lines is partially stained with black or brown pigments according to the color of the hilum. Reports of the relationship between seed mottling and virus transmission are inconsistent (12,14,18,19,20). Most reports concluded that mottling was an indication that the affected seeds came from SMV-infected plants or that they carried SMV (15,17,24,25,26).

In this study, 11 yellow-seeded soybean cultivars and lines were studied to determine if *(1)* mottled and nonmottled seeds produced SMV-infected plants, *(2)* SMV-infected plants produced mottled seeds, and *(3)* these cultivars and lines differed in rates of SMV seed transmission.

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MATERIALS AND METHODS

Eleven yellow-seeded soybean cultivars and plant introduction lines from the soybean germ plasm collection of Alabama A&M University were used. Three of the cultivars, Bragg, Essex, and Leflore, are among the most widely grown cultivars in the southeastern United States. Mottled and nonmottled seeds were produced in local field-grown plantings with natural infection by SMV. Three hundred mottled and 300 nonmottled seeds of each cultivar or line were selected. The seeds were planted 7 cm apart within rows and 15 cm apart between rows each time in a randomized block design in soil benches, 4.12 × 0.85 × 0.31 m, in the greenhouse. Seedlings were grown for 5–6 wk at 18±3 C. Symptoms of SMV were reported to be more intense on seedlings grown at this temperature than at higher temperatures (8,26). The numbers of plants with SMV symptoms from mottled and nonmottled seeds were recorded for each of three replicates and the data were combined for analysis of variance.

The SMV in soybean cultivars commonly grown in northern Alabama was determined according to the procedure of Cho and Goodman (6) to be strain SMV-G7. Antigen of an Alabama isolate of SMV-G7 was prepared by grinding 200 g of SMV-infected leaves of cv. Bragg in 300 ml of 0.02 M Tris-HCl buffer at pH 7.0 with 0.001 M 2-mercaptoethanol. The crude leaf extract was filtered through cheesecloth, and the virus was purified by differential centrifugation. The virus was further purified by sucrose density gradient centrifugation (4,5) and quantified spectrophotometrically at 260 nm.

Polyclonal antibody to the Alabama isolate of strain SMV-G7 was produced in rabbits during five weekly injections of 3–4 ml (1 mg/ml) SMV suspension followed by a booster injection of 4 ml at week 7 (2). Gamma globulin was obtained by ammonium sulfate precipitation (11), resuspended in half-strength phosphate buffered saline (PBS, containing 0.07 M NaCl, 0.001 M KH2PO4, 0.005 M Na2HPO4·7H20, 0.0015 M KCl) and quantified spectrophotometrically at 280 nm (1.4 OD =1 mg/ml). The gamma globulin was stored at −18 C. Leaves of symptomless soybean plants grown from mottled and nonmottled seeds were assayed for SMV using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with an alkaline phosphatase conjugate following the procedure of Hill (11). Negative control wells contained PBS. No other controls were used. Positive reactions indicating the presence of SMV in the sample were determined visually by the presence of faint yellow color.

Eight plants from each of four cultivars/lines, two SMV-infected and two SMV-free plants grown from mottled seeds, and two SMV-infected plants and two SMV-free plants grown from nonmottled seeds, were transplanted separately to Pro-mix potting medium (Premier Brand, Inc., Yonkers, NY) contained in 25-cm-diameter plastic pots and grown to maturity in the greenhouse. The number of mottled and nonmottled seeds produced by each plant was recorded. The plants in the greenhouse were sprayed every 2 wk with malathion to control aphids, whiteflies, etc. Four weeks before the seeds were harvested, virus-free plants in the greenhouse were again assayed by DAS-ELISA.

RESULTS AND DISCUSSION

The presence of SMV in soybean seedlings grown from mottled and nonmottled seeds did not differ significantly. Both mottled and nonmottled seeds produced SMV-infected seedlings. Leaves of SMV-infected plants were rugose or crinkled, mottled or curled downward like those described by Sinclair and Backman (26). However, the presence of SMV in soybean seedlings grown from various cultivars and lines varied significantly (Table 1, Fig. 1).

Cultivars/lines differed in their rates of SMV seed transmission (Fig. 1). The frequencies of seed transmission varied significantly from 25.7 to 91.7% from the combined virus infection of mottled and nonmottled seeds (Fig. 1). Virus-infected plants of each of the soybean cultivars and lines (Epps, Wright, PI 423900, and Essex) that were grown from mottled seeds produced an average of 71.5% mottled and 28.5% nonmottled seeds (Table 2). Virus-free plants grown from mottled seeds produced an average of 65.2% mottled and 34.8% nonmottled seeds (Table 2). Virus-infected plants grown from nonmottled seeds produced 81.9% mottled and 18.1% nonmottled seeds (Table 2). Virus-free plants grown from nonmottled seeds produced 70.8% mottled and 29.2% nonmottled seeds (Table 2). There was no apparent relationship between the virus condition of the plants in the first experiment and their origin from mottled or nonmottled seeds. Mottled and nonmottled seeds were selected from greenhouse grown plants that had natural seed infection of SMV. Similarly, in the second experiment both SMV-infected and SMV-free plants produced mottled seed. The results of this study agree with a previous report (12) that seed mottling cannot be considered an indicator of the rate of seed transmission of SMV in soybean seeds.

Table 1. Analysis of variance of soybean mosaic virus–infected seedlings grown from mottled and nonmottled seeds of soybean cultivars and lines

Source of variance	Degrees of freedom	Sum of square	*F* value	*Pr* > *F*
Replication	2	823.51	2.19	0.1240
Cultivar/line	10	32,386.55	17.26	0.0001
Treatment (mottled vs nonmottled)	1	118.94	0.63	0.4304
Cultivar/line × treatment	10	2,764.40	1.47	0.1834
Error	42	7,879.55		

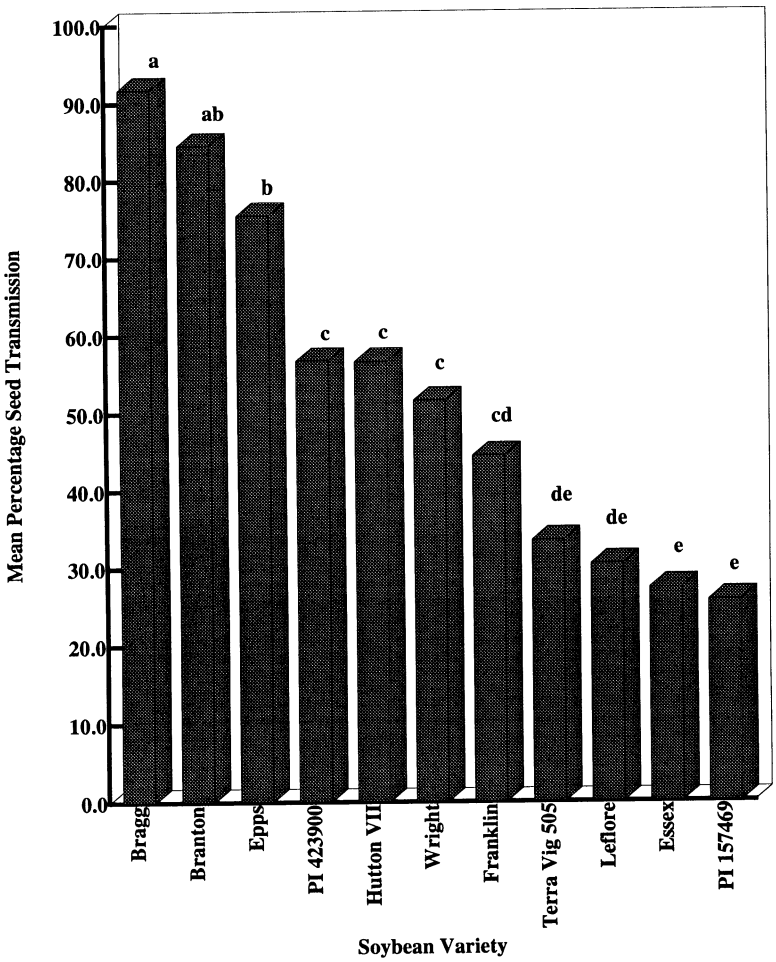

Fig. 1. Mean percentage of seed transmission from combined mottled and nonmottled seed in selected cultivars and lines (LSD; alpha = 0.05).

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Table 2. Number of mottled or nonmottled seeds produced by soybean mosaic virus (SMV)–infected and virus-free soybean plants grown from mottled and nonmottled seeds[a]

	Mottled seeds				Nonmottled seeds			
	SMV-infected plants		SMV-free plants		SMV-infected plants		SMV-free plants	
Cultivars/line	No. of MS[b]	No. of NMS[c]	No. of MS	No. of NMS	No. of MS	No. of NMS	No. of MS	No. of NMS
Epps	211	18	224	86	263	33	270	78
Essex	16	51	68	62	98	35	83	50
Wright	56	13	57	13	8	1	13	5
PI 423900	119	78	68	62	98	34	85	53
Mean (%)	71.5	28.5	65.2	34.8	81.9	18.1	70.8	29.2

[a] Seeds from two plants in each respective group were combined and counted.
[b] Mottled seeds.
[c] Nonmottled seeds.

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