Detection of Soybean Mosaic Virus in Seed by Solid-Phase Radioimmunoassay

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ABSTRACT

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Virus antigen content of soybean seeds from plants inoculated with soybean mosaic virus in field trials with 15 cultivars was determined by solid-phase radioimmunoassay. Antigen content of seeds did not vary significantly between plants inoculated at growth stages V-1 and R-2. Plants inoculated at stage R-2 produced seed with a greater percentage of seed coat mottling than those inoculated at stage V-1. The percentage of mottled seed was not correlated with virus antigen content of seeds in most cultivars; however, some cultivars had a high correlation. Yield loss was greater in plants inoculated at stage V-1 than at R-2 in 1978; in 1979, there was no significant difference. Weight of 100 seeds from inoculated plants was less than weight of 100 seeds from uninoculated plants. Seed weights did not vary significantly with inoculation time.

Primary inoculum of soybean mosaic virus (SMV) in Iowa consists of infected seedlings derived from SMV-infected seed (8). Because most soybean cultivars now grown in Iowa are susceptible to SMV, the most prudent control measure is to use seed containing no or low levels of SMV.

The association of seed coat mottling with seed transmission suggests an attractive way to certify seed for absence of virus, but unfortunately, this association is inconsistent (8,9,12) and depends on environment and genotype (4,12,14,15). Therefore, for most soybean cultivars, this trait is unreliable as a definitive criterion for absence of SMV in seed (8,12).

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Detection of virus antigen in seed by serologic testing provides another attractive method for seed certification. Recently, serologically specific electron microscopy and enzyme-linked immunosorbent assay have been used for detecting SMV in crude extracts of infected seed (2,10). We report here application of the solid-phase radioimmunoassay (SPRIA) system recently developed in our laboratory (3) for detection of SMV antigen in soybean seed. We also examined relationships between seed coat mottling and virus antigen content, yields, and seed weights in 15 soybean cultivars inoculated with SMV.

MATERIALS AND METHODS

Isolate Ia 75-16-1 of SMV used in these experiments has been described previously (6,7,11). Experiments were conducted at the Curtiss Experimental Farm near Ames, IA.

Fifteen soybean cultivars representing maturity groups I–iV were planted (one seed per 6.1 cm) on 5 May 1978 and 11 May 1979 in rows 6.1 m long and 76 cm apart. The experimental design was a split plot with two replicates in each of 2 yr. Cultivars were applied to the whole plots, consisting of eight rows, and SMV infection treatments were randomly allocated to split plots within each whole plot.

Plants in one row in each plot were inoculated with SMV at growth stage V-1 (5), and plants in another row were inoculated at growth stage R-2. Plants in a third row were left uninoculated as a control. Treatments were separated by a row of untreated soybeans.

Inoculum was prepared by triturating leaves of Williams soybeans inoculated 21 days earlier in 0.5*M* sodium-potassium phosphate buffer, pH 7.2. Carborundum (600-mesh) was added to the inoculum before it was used to mechanically inoculate plants.

Rows were harvested individually. Seeds were dried to uniform moisture, cleaned, weighed, and graded for seed coat mottling. The weight of 100 seeds per row was obtained as a measure of seed size.

The amount of virus antigen in seeds from each treatment was determined by SPRIA. Batches of 100 seeds were ground at setting no. 6 for 1 min in 100 ml of 0.05*M* sodium borate, pH 7.2, with a Brinkmann Polytron homogenizer (Brinkmann Instruments Inc., Westbury, NY 11590) and model no. PT 20 ST probe generator. Extracts were squeezed through cheesecloth, and 1-ml fractions were used for analysis as described elsewhere (3). Levels of SMV antigen in each sample were determined by linear interpolation from a standard curve relating ng SMV antigen to counts per minute established concurrently with each assay.

Data on yield, percentage of seeds with mottled seed coats, weight of 100 seeds, and ng virus antigen were analyzed with standard analysis of variance procedures, and treatment means were compared statistically. Correlation coefficients between seed coat mottling and antigen content were calculated.

RESULTS

SPRIA showed that the seed sample of the immune cultivar Marshall (11) contained no detectable SMV antigen (Table 1). In contrast, seeds from cultivars Clark 63 and Calland contained very high levels of virus antigen. Cultivars with intermediate antigen content were Steele, Hark, Hodgson, Corsoy, Beeson, and Williams, with Wells, Amsoy 71, and Harcor somewhat higher. Seeds of the

cultivars Wayne, Woodworth, and Cutler 71 contained relatively low amounts of virus antigen.

Antigen levels in seeds from inoculated plants were greater ($P < 0.03$) than those in seeds from uninoculated plants (Table 1). Antigen was detected, however, in seeds from the uninoculated plants, which suggests that SMV was spread, presumably by aphids (11), from inoculated to uninoculated rows. There were no significant differences in levels of antigen between seeds from plants inoculated at stages V-1 and R-2 or among maturity groups.

Incidence of seed coat mottling varied with cultivar and maturity group; mean values for early maturity groups generally were lower than those for later maturity groups (Table 1). Plants inoculated at stage R-2 generally produced seed with a higher incidence of seed mottling ($P < 0.01$) than those inoculated at stage V-1. Control plants produced fewer mottled seeds than those inoculated with SMV. Incidence of mottling was very low in the cultivar Marshall, while Amsoy 71, Harcor, Wayne, Calland, Cutler 71, and Clark 63 showed a high degree of mottling.

The correlation coefficient between percentage of seed coat mottling and SMV antigen content was highly significant ($P = 0.01$) for cultivars Steele, Hodgson, and Beeson, significant ($P = 0.05$) for cultivars Amsoy 71 and Williams, and nonsignificant for the remaining cultivars (Table 1). No correlation coefficient could be calculated for the cultivar Marshall because virus antigen was not detected in seed from this cultivar.

Yield loss induced by inoculation with SMV was greater in 1979 than in 1978 (Table 2). Loss was greater ($P < 0.01$) in 1978 for plants inoculated at stage V-1 than at stage R-2. In 1979, however, yield loss was not significantly different between the two inoculation times. There was no evidence that maturity groups responded differently to infection.

Weights of 100 seeds were lower ($P < 0.01$) for samples from inoculated plants than from uninoculated plants (Table 2). Time of inoculation did not significantly affect 100-seed weight values, however.

DISCUSSION

SPRIA revealed distinct differences in SMV antigen levels in seeds of the cultivars examined in this study. In contrast to the negative correlation between inoculation time and seed transmission of SMV, SMV antigen content in seeds does not vary significantly with inoculation time. Apparently, as suggested by Bowers and Goodman (1), SMV may be inactivated in some cultivars upon seed maturation, resulting in detection of antigen but not infectious virus. Unfortunately, although Bowers and Goodman studied the relationship between seed transmission and inoculation time in the cultivar Williams, they did not analyze the relationship between inoculation time and antigen presence.

The incidence of seed coat mottling was higher ($P < 0.01$) when plants were inoculated at stage R-2 than at stage V-1. This contrasts with the observation of Bowers and Goodman (1), based on experiments conducted for 1 yr with one cultivar, that inoculation time has no effect on incidence of seed coat mottling. Although seed coat mottling is viewed as a generally inconsistent and unreliable indicator for presence of SMV in seed (8,9,12), the correlation between the percentage of seed coat mottling and virus antigen content was significant for five cultivars in our study. Recognizing that a serologic test detects virus antigen and not infectious virus and that early

Table 1. Incidence of seed coat mottling and soybean mosaic virus (SMV) antigen content of seeds from soybean cultivars inoculated at two growth stages with SMV

		Growth stage when inoculated[a]						
		Uninoculated[a]		V-1		R-2		
Maturity group	Cultivar	Mottling (%)	ng SMV antigen	Mottling (%)	ng SMV antigen	Mottling (%)	ng SMV antigen	Corr. coeff.[b]
I	Steele	2.1	2,650	18.2	11,950	26.5	7,600	0.80[c]
I	Hark	4.1	5,750	30.5	11,075	31.2	6,300	0.14
I	Hodgson	3.5	1,650	6.7	4,425	28.5	20,425	0.99[c]
II	Wells	4.3	8,250	21.7	8,875	46.0	18,125	0.12
II	Corsoy	3.5	7,925	19.0	3,125	22.5	5,100	0.08
II	Amsoy 71	7.0	5,500	44.7	17,575	46.2	9,175	0.70[d]
II	Marshall	1.5	0	1.7	0	3.0	0	...
II	Harcor	4.7	4,825	31.2	13,650	43.2	13,700	0.33
II	Beeson	2.7	3,925	7.0	8,875	18.2	11,000	0.73[c]
III	Wayne	9.8	2,075	34.0	4,450	39.7	5,150	0.57
III	Woodworth	9.6	0	32.2	4,625	29.5	1,750	0.56
III	Calland	15.6	7,975	27.5	10,900	42.0	20,500	0.24
III	Williams	13.6	1,825	22.5	11,775	33.0	9,650	0.69[d]
IV	Cutler 71	16.6	2,250	26.2	5,050	42.7	4,425	0.36
IV	Clark 63	15.8	1,600	30.7	36,550	40.2	13,300	0.23

[a] Data are mean values of two replicates for each of 2 yr; differences between years were not significant ($P = 0.05$).
[b] Correlation coefficient of virus antigen content with percentage of seed coat mottling.
[c] $P = 0.01$, 10 degrees of freedom.
[d] $P = 0.05$, 10 degrees of freedom.

Table 2. Yield reduction and 100-seed weight of 15 soybean cultivars in four maturity groups inoculated at two growth stages with soybean mosaic virus

			Growth stage when inoculated[a]							
	Uninoculated[a]		V-1				R-2			
	100-seed weight (g)		100-seed weight (g)		Yield reduction (%)		100-seed weight (g)		Yield reduction (%)	
Maturity group	1978	1979	1978	1979	1978	1979	1978	1979	1978	1979
I	15.6	17.4	15.5	16.3	14	25	15.7	16.3	0	32
II	17.9	17.7	17.4	16.8	15	28	17.1	16.9	7	24
III	19.0	16.3	18.7	16.4	24	21	19.1	15.9	9	21
IV	19.4	15.0	19.0	14.6	27	24	19.2	15.2	8	29

[a] Data are mean values from two replicates for each year.

infection of soybeans generally results in seed with higher levels of SMV transmission (1,14), we believe that our data suggest that a seed producer might use seed coat mottling as a reliable indicator of potential virus seed transmission in a few cultivars such as Hodgson ($r = 0.99$). However, for most cultivars (eg, Corsoy [$r = 0.08$]), seed coat mottling is an unsuitable criterion for predicting presence of SMV in seed. Therefore, it is critical that a seed producer be aware of mottling differences in cultivars infected with SMV.

Inasmuch as infected soybean seed seems to provide the primary inoculum source for SMV in the upper Midwest (8), we believe that a quantitative measure of virus antigen in a seed lot may give seed producers a way to establish tolerance limits for distribution of seed lots containing SMV-infected seed to the commercial market. Although SPRIA measures virus antigen content rather than infectious virus, research in progress suggests that satisfactory guidelines for seed antigen levels, beyond which planting may be unwise, can be established.

Weight of 100 seeds, significantly less in samples from inoculated plants than in samples from uninoculated plants, reflected reduced seed size caused by infection of plants with SMV. Contrary to the data of Ross (14), seed size was reduced at both early and late inoculation dates.

Yield loss figures in 1978 substantiated the report of Ross (14) that losses are greater when inoculation times are early. Similar differences were not noted in 1979, when inoculation time did not significantly affect yield loss, as reported by Quiniones et al (13). Evidently, the effect of inoculation time on yield loss is highly dependent on environmental factors.

The yield loss estimates, based on yield comparisons with uninoculated plants and the supposition that most plants in an inoculated row are infected, may be conservative. The detection of virus antigen in seeds from control plants, which were not mechanically inoculated, indicated that some virus spread occurred in these plots. The effect on yield of these plants is unknown.

Loss estimates based on yield comparisons between plots in which all plants are inoculated and plots of uninoculated plants can represent only the maximum loss induced by a virus. Rarely does incidence of SMV in a commercial soybean field in Iowa approach 100%. Therefore, yield losses that we report probably are not representative of losses experienced in commercial soybean production. Research is in progress to develop technology for more realistic assessment of yield losses in relation to SMV seed antigen.

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