



Seed Coat Mottling in Food-Grade Soybean Cultivars in Response to Infection with BPMV and SMV.

Katy Martin Rainey¹, Sue A. Tolin², Ashlina Chin¹ and Heather Taylor¹,

(1) Crop & Soil, Environmental Sciences, (2) Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA



Abstract

In 2008 74 entries (MG V and VI) in a regional food-grade large- and small-seeded soybean cooperative yield test were planted in three blocks with a SMV inoculated treatment, BPMV inoculated treatment, and an uninoculated treatment, and seeds were harvested at maturity. We characterized foliar and seed coat virus symptoms, and analyzed correlations between observable symptoms and seed traits. Thirty-four lines were resistant to SMV, and all lines were susceptible to BPMV with variable symptom severity. Lines VS03-622 (Tomahomare x V81-1603), VS03-709 (V81-1603 x Pella), and TC02AXB-717 (N95-7440 x N7101) were resistant to SMV, and had very few percent mottled seeds in the BPMV treatment. Mean percent of mottled seeds was 59.86% in the BPMV treatment, 22.2% among susceptible lines in the SMV treatment, and 2.4% in the uninoculated treatment. Virus treatments significantly reduced seed quality but not seed size compared to the uninoculated treatment. Hilum color among lines ranged from yellow, to buff, to brown, to black but was not a significant source of variation for percent of mottled seeds, or degree and color of seed mottling. Seed size was not correlated with seed mottling symptoms. Severity of seed mottling was not correlated with foliar symptoms for either virus treatment, or both considered together. Resistance to SMV, or reduced SMV seed mottling and foliar symptoms among susceptible lines, were not associated with reduced BPMV seed mottling. Resistance or susceptibility to SMV foliar symptoms was a significant (P<0.05) source of variation for BPMV foliar symptoms.

Introduction

Soybean mosaic virus (SMV) and bean pod mottle virus (BPMV) are two important soybean diseases targeted by soybean breeders because they decrease yield and seed quality. Both viruses are a problematic for food-grade soybeans because they cause seed coat mottling (Figure X). In the mid-Atlantic growing region in 2007, some harvested lots of food-grade soybeans were unusable due to seed coat mottling, presumably from virus infection. Anecdotal evidence suggests BPMV incidence has increased in the same region in recent years.

Food-grade soybeans have light-colored hilums- brown, light brown, buff, tan, and yellow are acceptable. There are no reports on the effect of hilum color on seed coat mottling in soybean. Hobbs et al. (2003) evaluated eight soybean lines for reaction to both SMV and BPMV and reported that streaks emanating from the hilum were less apparent in light- as opposed to dark- hilum seeds. In the same study, they report that the non-seed-coat-mottling gene (Im) was infective against BPMV or SMV, and that the SMV-resistance gene Rsv1 is effective against mottling caused by SMV but not BPMV. The authors also observed a significant virus, line, and virus-line interaction for seed coat mottling, suggesting some soybean lines may be generally resistant to seed coat mottling caused by viruses.

There is no known resistance to BPMV in soybean, though some tolerant lines have been identified by reduced virus antigen levels (Hill et al., 2007). In order to identify soybean lines with reduced seed coat mottling in response to BPMV, it would be useful to conduct seedling germplasm screens for reduced foliar symptoms, but there are no reports of correspondence between foliar symptom severity and resistance to seed coat mottling in response to infection by either SMV or BPMV. Hill et al. (2007) report that extent of seed coat mottling and foliar symptom severity are not indicators of SMV or BPMV tolerance as measured by virus antigen levels.

Objectives

- Characterization of large- and small-seeded soybean lines in a regional food-grade test for foliar and seed coat virus symptoms.
- To determine if lighter hilum colors were associated with reduced seed coat mottling in response to virus infection.
- To determine if differences exist by line for reduced seed coat mottling in response to infection with BPMV and SMV.
- To characterize the effects of virus infection on seed quality and seed size in large- and small-seeded soybeans.

Materials and Methods

Germplasm

Maturity group V and VI large- and small-seeded food-grade soybean cultivars and experimental lines were used in this experiment, along with appropriate high-yielding checks (5002T, R04-42, 5601T, NC-Raleigh, NC-ROY), and SMV resistant (York) and susceptible (Lee) controls. Sources were Virginia Tech, Univ. Arkansas, Virginia State Univ., Univ. Tennessee, and North Carolina State Univ. 44 lines were large-seeded and 24 lines were small-seeded.

Experimental design

The experiment was planted May 2008. Twenty seeds per line were planted in rows three feet long with three feet between each row in three unreplicated blocks. Entries were randomized within blocks. The inoculated block was separated from the virus blocks by approximately 125'. Virus blocks were separated by a tier of SMV-resistant border.

Disease inoculation

Soybean seedlings in virus treatment blocks were inoculated three weeks after planting at the V1 growth stage. Virus strains SMV G1 and an unidentified but virulent strain of BPMV collected in Virginia were used. Inoculum were provided by Dr. Sue Tolin. Inoculum of each virus was prepared from freshly ground leaf tissue of virus-infected plants in 0.05M sodium citrate buffer with carborundum powder added. The abaxial side of one unifoliate was inoculated with an airbrush paint gun.

Data collection

Foliar virus symptoms were rated on a 0-5 scale in both inoculated blocks a few weeks after inoculation, where 0= no symptoms and 5= severe foliar chlorosis and rugosity. Seeds were harvested at maturity from all rows. Seed size was recorded as grams per 100 seeds. The number of mottled seeds in a 100-seed sample was recorded as percent mottled seeds. Rating scales were developed for seed quality (1-5), degree of mottling (1-6), and color of mottling (0-3). Seed quality scale accounted for seed coat luster, cracking, and seed coat discoloration due to fungal pathogens. Degree of mottling was based on the overall percentage of seed coats in a 100-seed sample covered with a mottle, with a 1 given for no mottling, a 2 for a trace amount of mottling, 3 for up to a quarter of the seed coat mottled, 4 for 25-50% of the seed coat mottled, 5 for 50-75% of the seed coat mottled, and 6 for 100% mottled (Fig. 1). The color of mottling scale was: 0= no mottling at all, 1= lightest shade of brown mottling, 2= medium shade of mottling, and 3= darkest shade of mottling.

Data analysis

The SAS program was used for statistical analysis using PROC CORR, PROC GLM, and PROC MEANS. When needed, analyses requiring normally-distributed data were conducted without data from SMV-resistant lines.

Results and Discussion

Virus disease data and seed quality are summarized in Tables 1 and 2. Thirty-five of the lines showed no foliar virus symptoms in the SMV treatment and only 1-2% mottled seeds, and thus were resistant to SMV. All lines were susceptible to BPMV. Lines TC05AXB-809, VS03-709, TC02AXB-717, and TC05AXB-810 were relatively tolerant to BPMV with mild foliar symptoms, and less than 10% mottled seeds. The BPMV treatment produced more severe seed mottling than the SMV treatment, with 37.5% more mottled seeds, and higher mean degree of mottling and lower quality scores. More severe foliar virus symptoms were observed in the SMV treatment compared to BPMV treatment (score of 2.3 vs. 1.9).

Sources of variation

ANOVA across treatments did not include data from SMV-resistant lines. Treatments were a significant source of variation at the P<0.0001 level for seed quality and size, percent mottled seeds, and degree and color of seed mottling, but was not significant for foliar virus symptoms. When considered across virus treatments only, line was not a significant source of variation for seed quality, percent mottled seeds, and degree and color of seed mottling but was significant at the P<0.05 level for foliar virus symptoms. Seed size class and maturity class had no effect on virus symptoms.

Effect of virus treatments on seed quality and size

Virus treatments significantly reduced seed quality when compared to the uninoculated treatment, but not seed size (Tables 3-6.) Mean seed size for large-seeded entries was significantly larger in the uninoculated block (mean 20.0 g/100 sds) versus the SMV treatment (mean 18.9 g/100 sds, P<0.05), and the BPMV treatment (mean 17.8 g/100 sds, P<0.0001). Mean seed size for small-seeded entries was not reduced by virus treatment.

Correlation of virus symptoms

Tables 7 through 9 show correlation of virus symptoms within and across virus treatments. In general, percent mottled seeds and degree and color of seed coat mottling and seed quality were all highly correlated. Foliar symptoms were not associated with seed mottling symptoms or seed quality across virus treatments (Table 7) or within virus treatments (Table 8 and 9).

Resistance to seed mottling

There was no correlation of seed mottling symptoms between virus treatments, i.e. lines were not identified with low seed coat mottling scores in both virus treatments. Table X shows correlations of virus symptoms between treatments.

SMV resistance and BPMV symptoms

Resistance or susceptibility to SMV was classified by foliar symptom scores and percent mottled seeds. Resistance or susceptibility to SMV did not contribute to variation in seed mottling symptoms in the BPMV treatment. Foliar BPMV symptoms were significantly reduced (P<0.05) among the SMV resistant lines as compared to SMV susceptible lines (Table 10.)

Effect of hilum color and seed size on mottling

Hilum color among lines ranged from yellow, to buff, to brown, to black but was not a significant source of variation for percent of mottled seeds, or degree and color of seed mottling. Seed size was not correlated with seed mottling symptoms.

Conclusions

- Use of SMV resistance genes in the best method to eliminate SMV-induced seed coat mottling in food grade soybeans.
- Lines resistant or tolerant to foliar and/or seed coat mottling SMV did not display reduced BPMV-induced seed coat mottling.
- The only way to identify lines with reduced BPMV-induced seed coat mottling may be to screen directly for the trait.
- Light hilum color did not reduce virus-induced seed coat mottling.
- In this study, virus infection reduced seed size in large-seeded soybeans.

Table 1. Summary of virus disease symptoms for SMV and BPMV treatments

	n	X ± (SD)	Min	Max
SMV				
foliar score	38	2.3 ± (1.0)	0.5	6.0
% mottle	39	22.3 ± (29.9)	0.0	100.0
degree of mottling	40	3.1 ± (1.5)	1.0	6.0
mottling color	40	1.9 ± (1.2)	0.0	3.0
BPMV				
foliar score	68	1.9 ± (0.7)	1.0	3.0
% mottling	70	59.8 ± (30.3)	0.0	100.0
degree of mottling	70	3.6 ± (1.4)	1.0	6.0
mottling color	70	2.1 ± (0.9)	0.0	3.0

Table 3. Mean SMV quality score compared to uninoculated treatment

	n	Mean	Std. Dev.	t-Stat	Pr > t
SMV	74	2.7	1.0	2.28	0.02
UNINOC	72	2.4	0.8		

Table 5. Mean SMV seed size score compared to uninoculated treatment

	n	Mean	Std. Dev.	t-Stat	Pr > t
SMV	72	15.0	6.1	-0.58	0.56
UNINOC	72	15.6	6.2		

Table 2. Summary of seed traits for all treatments.

	n	X ± (SD)	Min	Max
seed quality				
SMV	40	2.9 ± (0.9)	1.0	5.0
BPMV	70	3.4 ± (1.0)	1.0	5.0
UNINOC*	70	2.5 ± (1.0)	1.0	4.5
seed size				
SMV	39	13.5 ± (5.4)	6.3	25.8
BPMV	70	14.0 ± (5.4)	5.0	31.1
UNINOC	70	14.49 ± (6.1)	6.9	24.9

*UNINOC= data from uninoculated block.

Table 4. Mean BPMV quality score compared to uninoculated treatment

	n	Mean	Std. Dev.	t-Stat	Pr > t
BPMV	70	3.4	1.0	2.28	0.02
UNINOC	72	2.4	0.8		

Table 6. Mean BPMV seed size score compared to uninoculated treatment

	n	Mean	Std. Dev.	t-Stat	Pr > t
BPMV	70	14.1	5.4	-1.61	0.11
UNINOC	72	15.6	6.2		

Table 7. Correlation of seed coat mottling traits and foliar symptom severity across BPMV and SMV treatments

	Degree of mottling	Mottling color	Foliar Symptom Score	Seed quality
% mottle	***	***	n/s	***
Degree of mottling		***	n/s	***
Mottling color			n/s	***
Foliar symptom score				n/s

*, **, ***: Significant at the 0.05, 0.001, 0.0001 significance level, respectively. n/s = not significant

Table 8. Correlation of disease symptoms within SMV treatment

	Seed size	Percent mottling	Degree of mottling	Mottling color	Foliar symptom score
Quality	n/s	***	n/s	n/s	n/s
Seed size		n/s	n/s	*	n/s
Percent mottling			*	**	n/s
Degree of mottling				***	n/s
Mottling color					n/s

Table 9. Correlation of disease symptoms within BPMV treatment

	Seed size	Percent mottling	Degree of mottling	Mottling color	Foliar symptom score
Quality	***	***	***	***	n/s
Seed size		*	n/s	n/s	n/s
Percent mottling			**	**	n/s
Degree of mottling				***	n/s
Mottling color					n/s

Table 10. Mean BPMV foliar score for lines resistant or susceptible to SMV

Group	n	Mean	Std. Dev.	t-Stat	Pr > t
RES*	31	1.7	0.6	-2.04	0.05
SUS	37	2.0	0.7		

*RES= SMV resistant = foliar score of 0;

SUS= SMV susceptible = foliar score of 1 or greater.



Fig. 1. Degree of seed coat mottling rating scale.

Thank you to Dr. Tadesse Mebrahtu, Dr. Tommy Carter, and Dr. Pengyin Chen for cooperation in the regional food-grade soybean yield test.

References

- Hill, J.H. N.C. Koval, J.M. Gaska, and C.R. Grau. (2007). Identification of field tolerance to bean pod mottle and soybean mosaic viruses in soybean. Crop Sci. 47:212-218.
- Hobbs, H.A., G.L. Hartman, Y. Wang, C.B. Hill, R.L. Bernard, W.L. Pedersen, and L.L. Domier. 2003. Occurrence of seed coat mottling in soybean plants inoculated with Bean pod mottle virus and Soybean Mosaic virus. Plant Disease: 87:1333-1336.