Inheritance of Beet Mosaic Virus Resistance in Sugarbeet

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ABSTRACT

An annual line of sugarbeet (*Beta vulgaris*) was shown to be a source of beet mosaic virus (BMV) resistance. Inheritance studies indicated that one incompletely dominant gene, *Bm*, conditioned resistance. Local lesion counts on *Chenopodium amaranticolor* from BMV-infected sugarbeet sources showed that the concentration of virus was reduced by the *Bm* gene and that the heterozygous F_1 was intermediate in concentration. *Bm* was specific against all isolates of BMV tested, but appeared to have no modifying effect on systemic infection by other beet viruses.

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Beet mosaic virus (BMV) occurs in most areas of California where sugarbeet (*Beta vulgaris* L.) is grown. Bennett (1), Shepherd & Till (5), and Shepherd et al. (6) have shown that it occurs as a number of variants which differ widely in effect on sugarbeet yields. In field inoculation trials, root yield losses have varied from 5.9 to 20.4% (4, 5), depending upon the age of the plant when infection began and virus strain virulence. BMV infection is commonly associated with virus yellows and its pathogenic effects are additive to those caused by virus yellows (4). Bennett (1) suggested that damage caused by BMV may be increasing in California due to increasing prevalence of highly virulent strains. BMV is transmitted by the green peach aphid [*Myzus persicae* (Sulzer)] and is easily transmitted in the laboratory by mechanical inoculation.

The inheritance of resistance in sugarbeet to BMV has not been studied, although over the years symptom differences in strains of beets have been observed. Feltz & Marx (2) demonstrated by line selection and breeding that beet mosaic resistance was genetically controlled but did not determine how the resistance in their lines was inherited. A breeding line of sugarbeet (subsequently designated "8500") that appeared to be resistant to BMV (J. S. McFarlane, *personal communication*) was observed at Salinas, California. This study was undertaken to determine the inheritance of resistance in that line.

MATERIALS AND METHODS.–Sugarbeet breeding line 8500 (self-fertile, homozygous, annual) and its cytoplasmic male-sterile equivalent, 8500 cms, were used as the BMV-resistant parents. The homozygous diploid, self-fertile "C5600" line (3) was used as the susceptible parent in initial crosses. Testcross seed was obtained by backcrossing both parents to the F_1 cms (8500 cms × C5600). F_2 and F_3 populations were derived by selfing from reciprocal crosses between 8500 and C5600. Other lines of sugarbeet used as susceptible parents were crossed with 8500 using either self-sterility, Mendelian male sterility, or marker genes to insure obtaining the appropriate cross.

The parent, F_1, and segregating populations were grown in greenhouses in sterilized sand-soil mixture. Seedlings germinated in sand flats were transplanted and grown in 6-inch clay pots, four plants per pot.

Seedling beets were mechanically inoculated with BMV in the two-to-four true leaf stage. The BMV source was maintained in sugarbeet. Leaves or leaf tissue were macerated in buffer solution (0.02 M phosphate, pH 7, containing 0.02 M sodium sulfite) and the juice applied to carborundum-dusted leaves. A strain of BMV obtained from Shepherd et al. (6) that caused a severe, necrotic reaction on *B. vulgaris* was usually used, but other strains characterized by milder symptoms were also employed.

Systemic symptoms were first observed 7 to 10 days after inoculation. Thereafter, plants were scored at 1- or 2-day intervals. Three disease ratings or infection-type classifications were used: R (resistant) = no early evidence of systemic infection to isolated chlorotic spots on later-developing leaves; I (intermediate) = systemic symptom expression delayed by 4 to 7 days compared to the susceptible plants, then appearing as isolated chlorotic spots that do not coalesce or do not coalesce until the plants become chronically infected; S (susceptible) = first plants to show early symptoms of systemic infection, more severe reaction than R or I, with stunting, blistering, and vein-clearing. Susceptible plants have symptoms typical of those described by Bennett (1) and Shepherd et al. (6). When only two disease ratings were used to score the segregating populations, R and I were combined into one resistant class.

The segregating populations were tested for goodness of fit to classical Mendelian ratios using X^2 statistics.

The relative concentration of infective particles in the resistant and susceptible parents and their segregating populations was assayed using local lesion counts on *Chenopodium amaranticolor* Coste & Reyn. In test 1, BMV was obtained from immature leaves of chronically infected plants of 8500, C5600, and their F_1 progeny. Each gram of beet leaf tissue was diluted with 3.5 ml of 0.02 M phosphate buffer (pH 7 and containing 0.02 M sodium sulfite) and macerated with a mortar and pestle to obtain the inoculum mixture called "juice". A randomized block design was used in which three adjacent leaves were inoculated per replication. The juice source from each population was assigned at random to two of the six half-leaves and the total lesion count was used as a single observation. Fifteen replications of four plants were used. Analysis of variance was used to determine differences in lesion counts from the three sugarbeet sources.

In test 2, paired comparisons were made between the parental, F_1, and segregating populations. From recently matured leaves of chronically infected beets in the greenhouse, a No. 6 cork borer was used to randomly sample leaf tissue from representative plants of each population. The leaf tissue was diluted with 5 ml of buffer per gram, macerated with a mortar and pestle, and applied to carborundum-dusted leaves. For each inoculation pair, the two BMV juice sources were randomly inoculated onto the right or left half of each leaf of one plant. The *t-test* was used to determine significant differences in the number of lesions.

RESULTS.–No difficulty was experienced in obtaining systemic infection in the seedling beets using juice from beet. The few escapes that did occur were pulled. Escapes could be detected by the absence of local lesions on the inoculated leaves and the absence of systemic symptoms at the stage when they should have shown chronic infection. Local lesions produced on line 8500 and the resistant segregates were usually chlorotic. On line C5600 and the susceptible segregates, the local lesions were usually distinct chlorotic spots which turned necrotic and often caused premature senescence of the leaf.

The results of the 8500 × C5600 crosses are summarized in Table 1. These data indicate that BMV resistance from 8500 is inherited as a single dominant

TABLE 1. The infection-type distributions of sugarbeet lines 8500 and C5600 and their progeny 10 to 14 and 20 to 30 days after inoculation with beet mosaic virus

Parents and progeny	No. of tests	No. of plants[a] R	I	S	Ratio	X^2	*P* value
10-14 days							
8500	10	179	0	0			
8500 cms[b]	4	71	1	0			
C5600	10	0	0	169			
F_1 & F_1 cms	6	82	9	0			
F_1 cms × 8500	3	130	4	0			
F_1 cms × C5600	2	49	2	50	1R:1S[c]	0.01	.90-.95
F_2 (8500 × C5600)	2	132	2	37	3R:1S[c]	1.03	.25-.50
F_2 (C5600 × 8500)	2	158	1	36	3R:1S[c]	4.45	.03-.05
20-30 days							
8500	6	106	7	0			
8500 cms	4	61	10	0			
C5600	6	0	0	115			
F_1 & F_1 cms	6	2	105	0			
F_1 cms × 8500	3	55	79	0	1R:1I	4.30	.03-.05
F_1 cms × C5600	3	0	84	92	1I:1S	0.36	.50-.75
F_2 (8500 × C5600)	3	50	167	84	1R:2I:1S[d]	11.30	<.005
F_2 (C5600 × 8500)	3	86	215	92	1R:2I:1S[d]	3.67	.10-.25

[a] S (susceptible) = systemic symptoms with stunting, blistering, and vein-clearing; I (intermediate) = systemic symptom expression delayed by 4 to 7 days and less severe than susceptible reaction; R (resistant) = very mild expression of systemic symptoms.

[b] cms = cytoplasmic-male-sterile strain.

[c] X^2 calculated by combining R and I into one class.

[d] *P* values are .10-.25 and .25-.50 for the reciprocal F_2's when segregation is fitted to a 3R:1S ratio by combining R and I classes.

or incompletely dominant gene. During the incipient stages of symptom expression (10 to 14 days after inoculation), C5600 and other susceptible checks usually showed 100% infection, and the F_2 fits a 3R (resistant):1S (susceptible) ratio. The F_1 and testcross to 8500 expressed systemic symptoms similar to 8500. The testcross to C5600 fits a 1R:1S ratio. At this early stage of infection the parents and segregating populations fell into fairly discrete resistant and susceptible classes. This stage proved to be the best time to distinguish the susceptible homozygous-recessive class from the remainder of the segregating population.

At a later stage of infection (20 to 30 days after inoculation, depending upon time of year and greenhouse temperatures), the F_1 plants showed a level of systemic infection between the near absence of symptoms in 8500 and the severe symptoms in C5600 (Table 1). At this stage the F_2 populations approach a 1R:2I:1S ratio. About half the plants in the testcross to 8500 changed to a reaction similar to the F_1, and the populations nearly fit a 1R:1I ratio. The testcross to C5600 now fits a 1I:1S ratio.

The infection types in the segregating populations at this later date of infection showed somewhat less discrete classes. However, the plants classified earlier as susceptible continued to display the most severe symptoms, and the ones classified as resistant separated into two more-or-less distinct classes, resistant and intermediate. The F_2 populations showed a poor fit to a 1:2:1 ratio but continued to fit a 3:1 ratio.

At an even later stage of infection, the chronically infected plants in the greenhouse were very difficult to classify accurately. The resistant plants developed more severe symptoms. The susceptible plants partially recovered and the systemic symptoms in the newly developing leaves were less severe.

From one F_2 population, 76 plants were saved and selfed to develop F_3 lines. Seventy-one of these plants produced enough F_3 seed to be assayed for mosaic reaction. As expected for a single gene, the F_3 lines fit a 1:2:1 pattern. One-fourth were homozygous-resistant, one-half segregated 3R:1S, and one-fourth were homozygous-susceptible. The F_3 lines were compared to their F_2 parents. Of the 57 F_2 plants classified as resistant or intermediate, only one should have been classified as susceptible, and of the 14 plants classified as susceptible, only one was misclassified. Misclassification between the resistant and intermediate types occurred for about 20% of the F_2 plants.

In other crosses between 8500 and mosaic susceptible parents (Table 2), the F_2 populations fit the expected 3R:1S ratio and the testcrosses fit the expected 1I:1S ratio. This gene for BMV resistance from line 8500 is designated *Bm* (*Bm* = resistant, *bm* = susceptible).

Isolates of BMV from diverse locations were tested to determine the specificity of the *Bm* allele.

TABLE 2. Distribution of infection types for sugarbeet F_2 and testcross populations between resistant 8500 and susceptible parents after inoculation with beet mosaic virus

F_2 populations	No. of tests	No. of plants[a] R, I	No. of plants[a] S	Ratio	X_2	*P* value
8500 × 705	1	118	34	3:1	0.56	.25-.50
521 × 8500	1	119	39	3:1	0.01	.90-.95
712 × 8500	2	257	91	3:1	0.25	.50-.75
102 × 8500	3	693	225	3:1	0.12	.50-.75
Testcross populations		I	S			
102 × (102 × 8500)	4	342	309	1:1	1.67	.10-.25
mm[b] × (C5600 × 8500)	1	47	33	1:1	2.45	.10-.25
MM[c] × (C5600 × 8500)	4	429	439	1:1	0.12	.50-.75

[a] S (susceptible) = systemic symptoms with stunting, blistering, and vein-clearing; I (intermediate) = systemic symptom expression delayed by 4 to 7 days and less severe than susceptible reaction; R (resistant) = very mild expression of systemic symptoms.
[b] mm represents four monogerm parents used to initiate backcross series.
[c] MM represents six multigerm parents used to initiate backcross series.

Bm conditioned resistance to all isolates tested. However, slight variations in the resistant reaction occurred. Some differences in the susceptible reaction were also evident for these various isolates. The most severe isolates on the susceptible lines of beet were not always the most severe on 8500 and its resistant segregates. The opposite was also true; i.e., some less virulent isolates on susceptible lines of beet were more virulent on line 8500. These differences indicated that interactions occurred between genotypes of beet and virus.

This gene appears to be effective only against BMV. The systemic infection of an isolate of cucumber mosaic virus that readily goes to beet by mechanical inoculation was unaffected by the presence of the *Bm* gene. The systemic infection by other beet viruses (e.g., curly top virus) also appeared to be unaffected.

By comparing numbers of local lesions produced on *C. amaranticolor* by juice inoculation from various sugarbeet lines and infection type classifications, a correlation was obtained between symptom

TABLE 3. The number of beet mosaic virus (BMV) local lesions from sugarbeet on *Chenopodium amaranticolor* inoculated with juice from resistant 8500, susceptible C5600, and their progeny using half-leaf methods

BMV sources	No. of replications	Total lesions
Test 1		
8500	15	18 a[a]
F_1 cms[b] (8500 cms × C5600)	15	118 a
C5600	15	553 b
Test 2		
8500:8500 cms	7	1:6
8500:C5600	8	3:483**[c]
8500:F_1 cms	8	7:134**
8500 cms:F_1 cms	8	24:210**
8500:F_1 cms × 8500	8	10:203**
8500:F_1 cms × C5600	8	0:549**
8500:F_2 (8500 × C5600)	9	15:615**
8500:F_2 (C5600 × 8500)	9	1:352**
F_1 cms:C5600	9	180:1542**
F_1 cms × 8500:F_1 cms × C5600	9	140:879**
F_1 cms:F_2	9	188:245
(F_1 cms × 8500) R plants: I plants	6	41:112
(F_1 cms × C5600) I plants: S plants	7	787:954
(F_2) R plants: S plants	10	102:535**
8500:R plants (F_2)	11	7:27
S plants (F_2):C5600	10	240:617*

[a] Counts with a letter in common are not significantly different at the .01 level.
[b] cms = cytoplasmic-male-sterile strain.
[c] Paired counts with * and ** are significantly different at the .05 and .01 levels, respectively, according to the *t-test*.

expression and relative concentration or titer of infective particles of BMV. In test 1 (Table 3), juice from the susceptible line, C5600, produced a significantly greater number of lesions than the juice from the resistant line, 8500, or their F_1. The number of lesions from the F_1 was intermediate, although not significantly different from the resistant line.

In test 2 (Table 3), paired comparisons were made in various combinations. The difference in the number of local lesions between 8500 and its cytoplasmic-male-sterile equivalent, 8500 cms, was not significant. In other comparisons, juice from 8500 produced significantly fewer lesions than that from C5600, their F_1 generation, the testcrosses, or the F_2's. Juice from C5600 produced significantly more lesions than the F_1. In this test, comparisons between different pairs can not be made statistically, but, in general, the number of lesions produced by juice from various lines and populations was consistent with their expected genotypic differences for the *Bm* locus. The number of lesions was nearly proportional to the expected frequency of the resistant and susceptible alleles. This relationship was somewhat less clear when various phenotypic classifications of the segregating populations were compared with each other and with their parents. Juice from the resistant, intermediate, and susceptible classes of the segregating populations did not produce the sharp differences observed in the equivalent genotypes in the parents and F_1 generation.

DISCUSSION.–The allele *Bm* that conditions BMV resistance in beet does not confer immunity. Rather it appears that in some manner it restricts the multiplication or entry of the virus or infective particles and reduces their concentration. The reduced concentration is manifested in the plants by less severe symptom expression. This restriction is additive in the sense that the homozygous dominant genotype has a more restrictive influence than the heterozygous genotype and the heterozygous genotype is more restrictive than the homozygous recessive genotype.

Unlike many of the major field crops, few major genes are known which control pathogens in sugarbeet. *Bm* is one of the few major genes that has been identified in the *B. vulgaris* germplasm. With very few exceptions, beet breeders have had to rely upon repeated selections to obtain varying levels of quantitatively inherited resistance.

The relationship between sugar yield loss in sugarbeet caused by BMV infection and the presence of none, one, or both resistant alleles at the *Bm* locus is not known. *Bm* from annual 8500 is being backcrossed into biennial sugarbeet. These near-isogenic susceptible and resistant lines will be used in field studies to determine the differences in yield.

In addition to the segregation caused by the major gene from 8500, it was observed that variations in the degree of susceptibility occurred within some segregating populations and between some susceptible lines. These variations probably reflect the presence of minor genes that modify the effect of the major gene and that can also condition moderate levels of resistance in the absence of the major gene. Usually it was observed that lines possessing moderate levels of virus yellows resistance (beet yellows and beet western yellows viruses) were more resistant to BMV than virus yellows susceptible lines. These yellows-resistant lines often required several days longer to develop moderately severe symptoms than their more yellows-susceptible equivalents. The relationship between yellows resistance and beet mosaic resistance is being investigated.

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