# Inheritance of Nonnecrotic Resistance to Southern Bean Mosaic Virus in Cowpea

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## ABSTRACT

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Inheritance of nonnecrotic resistance to the cowpea strain of southern bean mosaic virus (SBMV) was determined in cowpea. Crosses between a cowpea line (California Blackeye) susceptible to SBMV and three resistant lines (Early Pinkeye, Iron, and PI 186465) and crosses among the three resistant lines were evaluated for virus concentration and symptoms of individual plants of $F_1$ and $F_2$ generations. $F_3$ plants of the crosses were evaluated for symptoms. Virus concentration was determined by absorbance readings at 260 nm of purified virions or by enzyme-linked immunosorbent assay. The moderate resistance of Early Pinkeye was conferred by a single gene with partial dominance for resistance. Resistance in Iron appears to be controlled by multiple genes with incomplete dominance. The extreme resistance of PI 186465 was largely controlled by one gene with partial dominance for resistance; however, minor genes appeared to be operating and affecting virus concentration and symptoms in plants not homozygous for the major gene. Determination of virus concentration, rather than symptomatology alone, was indispensable for genetic interpretation of some crosses. Correlation of symptom severity with virus concentration in $F_2$ populations varied among crosses.

The cowpea strain of southern bean mosaic virus (SBMV) is a virus with a narrow host range, infecting cowpea and a few other leguminous genera and species (16). It is a beetle-transmitted, seedborne virus with small (25 nm) isometric particles. Geographic distribution of the virus includes the United States and western Africa (17).

Studies of inheritance of resistance to SBMV in cowpea have focused on resistance involving hypersensitive reactions. Brantley and Kuhn (3) found that the hypersensitive reaction of the cultivar Clay was controlled by a single dominant gene.

Other types of resistance to SBMV, with no necrosis involved, have been reported in cowpea (11,14). These have involved symptomless reactions of lines to the virus or mild mosaic reactions as opposed to severe mosaic, leaf deformation, and stunting reactions. Several cowpea genotypes with symptomless or mild mosaic reactions to SBMV have been shown to restrict virus accumulation to levels lower than those of susceptible cultivars (11).

The purpose of this study was to determine the pattern of inheritance of nonnecrotic resistance to SBMV in several cowpea lines with mild or no symptoms and with restricted virus accumulation. Both virus concentration (purified virions) and symptoms were used as criteria for evaluating progeny of crosses of resistant lines with a susceptible line and with each other.

## MATERIALS AND METHODS

**Virus isolate.** The cowpea strain (CP) of SBMV was maintained in cowpea cultivar California Blackeye. Periodically, cowpea genotypes Iron, Clay, and PI 399419 were inoculated to test for the expected reaction to strain CP (10).

**Plant manipulation.** Plants were grown in the greenhouse in a methyl bromide-fumigated 3:1:1:1 (v/v) mixture of soil-sand-vermiculite-perlite and fertilized weekly with 20-20-20 (NPK) soluble solution. Plants for breeding purposes were grown in 21-cm-diameter pots. Plants used in virus concentration and symptom studies were seeded at a rate of one per 10-cm pot for $F_1$ and $F_2$ generation plants and four per pot for $F_3$. Greenhouse temperatures ranged from 25 to 35 C in summer and from 20 to 30 C in winter.

Plants to be evaluated for virus concentration and/or symptoms were inoculated in the primary leaf stage (7–9 days after seeding) with purified virus inoculum (500 $\mu$g/ml in 0.01 M potassium phosphate [pH 7.0] containing 1% Celite) (11). Primary leaves were rubbed twice with a cheesecloth pad.

**Inheritance studies.** Four cowpea genotypes were selected to make crosses to evaluate the inheritance of resistance to SBMV. A

previous study (11) determined different levels of resistance based on virus concentration and symptomatology: California Blackeye, 1,000 μg or more of virus per gram fresh weight of leaf tissue, severe mosaic, leaf distortion, and stunting (susceptible); Early Pinkeye, 50–136 μg/g, mottle (moderately resistant); Iron, 8–30 μg/g, no symptoms but occasionally faint chlorotic spots (resistant); and PI 186465, 100 ng/g or less, symptomless (extremely resistant). Six crosses were made: California Blackeye × Early Pinkeye, California Blackeye × Iron, California Blackeye × PI 186465, PI 186465 × California Blackeye, Early Pinkeye × PI 186465, and Iron × PI 186465.

$F_1$ plants were allowed to self to produce seeds for $F_2$ plants. After evaluation of $F_2$ plants for virus concentration and symptoms, selected $F_2$ plants of crosses between susceptible California Blackeye and the three resistant lines were allowed to self to produce seeds for $F_3$ plants. $F_3$ plants (20 or more plants per line) of crosses between susceptible California Blackeye and the three resistant lines were evaluated for homogeneity or heterogeneity on the basis of symptomatology. For each cross, parents were included in the evaluation each time $F_1$, $F_2$, or $F_3$ plants were tested.

**Virus quantitation.** In some studies, inoculated primary leaves were harvested 18–28 days after inoculation from individual plants of parental, $F_1$, and/or $F_2$ plants. Virus was then isolated and quantified. In other tests, trifoliolate leaf tissue (usually one leaflet each from the second, third, and fourth leaves) from individual plants was used to determine virus concentration 23–40 days after inoculation of primary leaves.

Leaves weighing 1–5 g, from individual plants, were homogenized in 10 ml of 0.1 M sodium acetate buffer (pH 4.5) containing 0.02 M sodium bisulfite, 5 ml of chloroform, and 5 ml of butanol. After centrifugation at 10,000 *g* for 10 min, the upper aqueous layer was frozen at −20 C. Thawing was followed by low-speed centrifugation (10,000 *g* for 10 min), and virus in the resulting supernatant was concentrated by centrifuging at 165,000 *g* for 75 min. Pellets were suspended in 0.01 M potassium phosphate buffer (pH 7.0). The partially purified virus was analyzed spectrophotometrically (absorption coefficient = 5.85 $[mg/ml]^{-1}$ $cm^{-1}$ at 260 nm) (16).

Classes of plants with low concentrations of virus were particularly important to the interpretation of inheritance data in this study. Therefore, stringent tests were conducted to identify plants with less than 100 μg/g of virus. A second cycle of ultracentrifugation caused a significant loss of virus, particularly for samples with small quantities. The virus concentration of all samples was reduced by 13 μg/g; this is an average estimate for ultraviolet-absorbing host constituents found in uninoculated plants when they are treated to the purification procedure described. For some samples, the partially purified preparations were subjected to sucrose density gradient centrifugation, which allowed separation of virus and host constituents and could detect 1 μg of virus per plant sample (11). In other cases, plants were placed in classes based on enzyme-linked immunosorbent assay (ELISA) (4).

**ELISA.** Plants with no symptoms, or those with only chlorotic speckling as systemic symptoms, frequently were evaluated by double-antibody sandwich ELISA. The tissue was extracted directly in PBS (phosphate-buffered saline)-Tween-PVP (0.02 M potassium phosphate buffer, pH 7.3; 0.15 M NaCl, 0.003 M KCl; 0.05% Tween 20; 2% polyvinyl pyrrolidone) at a volume equaling 5× the weight of the issue. An absorbance at 405 nm of more than 2× the absorbance of extracts from healthy plants was considered a positive reaction.

**Symptom evaluation.** An index for systemic symptoms was developed for evaluating parents and progeny of crosses. The index was as follows: 0 = symptomless, 0.5 = slight chlorotic speckling, 1 = moderate chlorotic speckling, 2 = mild to moderate mosaic, 2.5 = moderate-severe mosaic with slight leaf distortion, 3 = severe mosaic with leaf distortion, and 4 = severe mosaic with severe leaf distortion. Parental evaluations were PI 186465, 0; Iron, 0–0.5; Early Pinkeye, 2.0; and California Blackeye, 4.0. No scale was developed for local reactions on inoculated leaves; the symptoms were simply recorded. Symptoms of the $F_1$ and $F_2$ plants of the crosses (along with parental lines) were evaluated at 2 wk for local reactions on the inoculated primary leaves and twice for systemic symptoms 3 and 4–8 wk after inoculation. A single $F_3$ symptom evaluation, for systemic symptoms only, was made 3–4 wk after inoculation.

## RESULTS

**Susceptible × moderately resistant.** The California Blackeye × Early Pinkeye $F_1$ was intermediate in virus concentration to the parents but closer to the resistant parent Early Pinkeye (Table 1). Symptom severity of the $F_1$ and Early Pinkeye were similar (rating of 2), although the mosaic patterns of the $F_1$ and of Early Pinkeye were distinguishable.

$F_2$ plants of the California Blackeye × Early Pinkeye cross could be classified into three groups on the basis of symptoms: Early Pinkeye-like (38 plants), $F_1$-like (80 plants), and California Blackeye-like (42 plants). Average virus concentrations of representative plants from the three symptom groups (Table 2) were similar to those of Early Pinkeye, $F_1$ and California Blackeye plants, respectively.

When allowed to self, Early Pinkeye-like $F_2$ plants produced all Early Pinkeye-like $F_3$ plants (Table 3). $F_1$-like plants all produced segregating $F_3$ lines. These lines produced a combined total of 118 Early Pinkeye-like plants, 239 $F_1$-like plants, and 118 California

TABLE 1. Virus concentration and disease reaction in four cowpea genotypes and $F_1$ and $F_2$ plants of six crosses

| | Disease reaction | | Virus concentration | |
|---|---|---|---|---|
| Cowpea genotype | Symptoms[a] | Severity rating[b] | Plants tested | μg/g[c] (av.) |
| California Blackeye | LC, Mo, D, St | 4 | 5 | 970 |
| Early Pinkeye | LC, Mt | 2 | 5 | 40 |
| $F_1$ | LC, Mo | 2 | 8 | 270 |
| $F_2$ | Variable | 2,4 | 160 | 392[d] |
| California Blackeye | LC, Mo, D, St | 4 | 12 | 1,160 |
| Iron | N, LC | 0–0.5 | 12 | 15 |
| $F_1$ | LC, Mo | 2.5 | 19 | 565 |
| $F_2$ | Variable | 0–4 | 176 | 597 |
| California Blackeye | LC, Mo, D, St | 4 | 12 | 1,380 |
| PI 186465 | N | 0 | 12 | <1 |
| $F_1$ | LC, Mo | 2 | 18 | 190 |
| $F_2$ | Variable | 0–4 | 123[e] | 186 |
| PI 186465 | N | 0 | 6 | <1 |
| California Blackeye | LC, Mo, D, St | 4 | 6 | 1,997 |
| $F_1$ | LC, Mo | 2 | 6 | 180 |
| $F_2$ | Variable | 0–4 | 165 | 297[d] |
| Early Pinkeye | LC, Mt | 2 | 12 | 136 |
| PI 186465 | N | 0 | 12 | <1 |
| $F_1$ | LC | 0.5 | 15 | 4 |
| $F_2$ | Variable | 0–4 | 166 | 149 |
| Iron | N, LC | 0–0.5 | 12 | 6 |
| PI 186465 | N | 0 | 12 | <1 |
| $F_1$ | LC | 0–0.5 | 12 | 6 |
| $F_2$ | Variable | 0–4 | 175 | 86 |

[a] Local symptoms: N = none, LC = local chlorosis. Systemic symptoms: D = distortion, Mo = mosaic; Mt = mottle, and St = stunting.

[b] Disease rating (systemic symptoms): 0 = symptomless, 0.5 = slight chlorotic speckling, 1 = moderate chlorotic speckling, 2 = mild to moderate mosaic, 2.5 = moderate-severe mosaic with slight leaf distortion, 3 = severe mosaic with leaf distortion, and 4 = severe mosaic with severe leaf distortion.

[c] Micrograms of virus per gram fresh weight of leaf tissue.

[d] Weighted average based on averages of representative plants of three symptom classes.

[e] Data from inoculated primary leaves. Twelve of 135 California Blackeye × PI 186465 $F_2$ plants had primary leaves which were either very small or damaged or had abscised at a time of evaluation, leaving 123 for sampling.

Blackeye-like plants, a nearly perfect 1:2:1 ratio (chi-square = 0.02, $P = 0.99$). California Blackeye-like $F_2$ plants produced all California Blackeye-like $F_3$ plants.

Chi-square evaluation of segregation of the $F_2$ population is presented in Table 4. An acceptable fit to a 3:1 ratio was obtained for the California Blackeye × Early Pinkeye cross, with the Early Pinkeye-like plants constituting about one-fourth of the $F_2$ population. Although the numbers of California Blackeye-like, $F_1$-like, and Early Pinkeye-like plants (42, 80, and 38, respectively) represent a statistically acceptable 1:2:1 ratio, for the purpose of presenting all crosses in a single table, the ratio was simplified to 3:1.

**Susceptible × resistant.** California Blackeye × Iron $F_1$ virus concentration was midway between that of the two parents (Table 1). Symptom severity of $F_1$ plants was also intermediate to that of the parents. Only one clear-cut $F_2$ symptom-virus concentration group could be distinguished in the California Blackeye × Iron cross: a group of 11 plants of the 176 total was similar to Iron in symptoms and virus concentration. The remainder of the $F_2$ represented a continuum of symptom expression from very mild to very severe. Virus concentration ranged from very low to very high (Table 2). Two other plants had virus concentration in the 1–100 μg/g (Iron) range but had more severe symptoms than Iron. This accounts for the 13 plants in the 1–100 μg/g range in Table 2.

Five of 11 Iron-like $F_2$ plants produced all Iron-like $F_3$ plants (Table 3). The other six Iron-like $F_2$ plants produced segregating $F_3$ lines. During the evaluation of the California Blackeye × Iron $F_3$ plants, late germination (caused by hard seed coat) of some seeds occurred, and some plants therefore escaped inoculation. The number of Iron-like $F_2$ plants yielding Iron-like $F_3$ lines could, therefore, have been lower than five, because a few uninoculated plants of some lines might have been misclassified as Iron-like (trifoliolate leaves of Iron were usually symptomless) rather than as more susceptible than Iron.

For the same reason, the number of segregating $F_3$ lines for the other three $F_2$ groups of the California Blackeye × Iron cross in Table 3 may not be accurate. The data from these three $F_2$ groups are useful mainly in demonstrating the complexity of the relationship between virus concentration and symptoms in this cross. It was possible to select a small group of eight $F_2$ plants with mild symptoms that had higher average virus concentration (825 μg/g) than a group selected for both severe symptoms and high virus concentration (646 μg/g). It was also possible to select an $F_2$ group with mild symptoms and relatively low virus concentration (191 μg/g).

When the correlation between numerical symptom values (0–4) and virus concentration was calculated for the entire California Blackeye × Iron $F_2$ population, a value of $r = 0.48$ was obtained.

TABLE 2. Segregation of $F_2$ progeny of cowpea crosses on the basis of concentration of southern bean mosaic virus

| Virus concentration (μg/g)[a] | Number of plants | | | | | |
|---|---|---|---|---|---|---|
| | CB × EP[b] | CB × IR | CB × 18 | 18 × CB | EP × 18 | IR × 18 |
| <1 | ... | 0 | 35 | 39[d] | 24 | 42 |
| 1–100 | 38[c] | 13 | 21 | ... | 72 | 115 |
| 101–200 | ... | 13 | 19 | ... | 41 | 9 |
| 201–300 | 80[c] | 13 | 16 | 83[d] | 11 | 4 |
| 301–400 | ... | 16 | 12 | ... | 9 | 3 |
| 401–500 | ... | 20 | 7 | ... | 3 | 1 |
| 501–600 | ... | 18 | 3 | ... | 1 | 0 |
| 601–700 | ... | 23 | 3 | ... | 2 | 1 |
| 701–800 | ... | 14 | 6 | 43[d] | 1 | 0 |
| 801–900 | ... | 11 | 4 | ... | 1 | 0 |
| 901+ | 42[c] | 35 | 9 | ... | 1 | 0 |
| Total no. | 160 | 176 | 135 | 165 | 166 | 175 |

[a] Micrograms of virus per gram fresh weight of leaf tissue. See Table 1 for parental levels of virus concentration in each cross.
[b] Abbreviations: CB = California Blackeye, EP = Early Pinkeye, IR = Iron, and 18 = PI 186465.
[c] Average virus concentration for eight plants each of EP-like (38), $F_1$-like (80), and CB-like groups of plants (42), respectively.
[d] Average virus concentration for eight plants each of 18-like (39), mild- to moderate-symptom (83), and severe-symptom (43) groups of plants, respectively.

TABLE 3. Reactions of $F_3$ progeny of crosses between California Blackeye and three resistant lines inoculated with southern bean mosaic virus

| Cross[a] | Reaction of $F_2$ plants | | $F_3$ plants from individual $F_2$ plants | | | |
|---|---|---|---|---|---|---|
| | Virus concentration (av. μg/g) | Disease reaction/ rating[b] | No. $F_2$ plants tested[c] | No. nonsegregating lines | No segregating lines | Range of symptoms in segregating lines |
| CB × EP (S × MR)[d] | 70 | MR/2 | 6 | 6 | 0 | ... |
| | 230 | −[e]/2 | 9 | 0 | 9 | 2,4 |
| | 990 | S/4 | 6 | 6 | 0 | ... |
| CB × IR (S × R) | <40 | R/0–0.5 | 11 | 5 | 6 | 0–2 |
| | 191 | −[e]/0.5–2 | 11 | 0 | 11 | 0–3 |
| | 646 | S/3–4 | 12 | 6 | 6 | 0–4 |
| | 825 | −[e]/0.5–2 | 8 | 1 | 7 | 0–3 |
| CB × 18 (S × ER) | <1 | ER/0 | 9 | 9 | 0 | ... |
| | 190 | −[e]/2–2.5 | 10 | 2 | 8 | 0–4 |
| | 730 | S/3–4 | 7 | 6 | 1 | 0–4 |

[a] CB = California Blackeye, EP = Early Pinkeye, IR = Iron, and 18 = PI 186465.
[b] Disease rating (systemic symptoms): 0 = symptomless, 0.5 = slight chlorotic speckling, 1 = moderate chlorotic speckling, 2 = mild to moderate mosaic, 2.5 = moderate-severe mosaic with slight leaf distortion, 3 = severe mosaic with leaf distortion, and 4 = severe mosaic with severe leaf distortion.
[c] Number of $F_2$ plants from which seeds were tested. Twenty or more $F_3$ plants were observed for each $F_2$ plant.
[d] Abbreviations: S = susceptible, MR = moderately resistant, R = resistant, and ER = extremely resistant.
[e] Group of plants does not fit any of four disease reaction categories (footnote d) based on virus concentration and symptoms.

Because a maximum of five of 176 California Blackeye × Iron $F_2$ plants were homozygous for the Iron-like reaction, the 63:1 ratio was tested and found satisfactory (Table 4).

**Susceptible × extremely resistant.** California Blackeye × PI 186465 and reciprocal cross $F_1$ plants were intermediate to the parents in virus concentration but closer to the resistant parent, PI 186465 (Table 1). Symptom severities of the two reciprocal cross $F_1$ plants were intermediate to those of the parents.

In both crosses, $F_2$ plants included a group of symptomless and therefore easily distinguishable PI 186465-like plants that had no or negligible virus accumulation (negative by ELISA) and constituted about one-fourth of the $F_2$ populations (Table 2). In both reciprocal crosses, $F_2$ plants with symptoms could be grouped roughly into mild to moderate symptom plants and severe symptom plants, which made up about one-half and one-fourth of the $F_2$ populations, respectively. Plants within those groups were not uniform in symptom severity, however, and precise classification of all $F_2$ plants with symptoms into the two groups was difficult. Table 2 presents virus concentration of individual $F_2$ plants of the California Blackeye × PI 186465 cross. Thirty-five of the $F_2$ plants were PI 186465-like, with less than 1 $\mu$g/g virus concentration (negative by ELISA) and no symptoms. The 100 remaining plants, all with significant virus accumulations ($>1$ $\mu$g/g positive by ELISA), had symptoms and were grouped into a mild to moderate symptom group of 68 and a severe symptom group of 32. Average virus concentration for the two groups was 190 and 730 $\mu$g/g, respectively. Although an arbitrary line could be drawn at 400 $\mu$g/g separating a group of 68 lower concentration plants from a group of 32 higher concentration plants, such a division would not correspond exactly to symptom data. Eight plants of each symptom group would be on the wrong side of the virus concentration line, although generally close to the line.

The correlation between numerical symptom values (0–4) and virus concentration in the California Blackeye × PI 186465 as a whole was calculated as $r = 0.78$.

In the PI 186465 × California Blackeye $F_2$, 39 plants were PI 186465-like ($<1$ $\mu$g/g virus concentration, negative by ELISA; symptomless) (Table 2). The 126 remaining plants, all with significant virus accumulation ($>1$ $\mu$g/g, positive by ELISA), had symptoms and were grouped into a mild to moderate symptom group of 83 plants and a severe symptom group of 43 (Table 2). Average virus concentration of representative plants from the two groups was 210 and 770 $\mu$g/g, respectively, very similar to that of the corresponding groups in the California Blackeye × PI 186465 $F_2$ population.

With the California Blackeye × PI 186465 cross, all nine PI 186465-like $F_2$ plants tested bred true for the PI 186465-like reaction (Table 3). Eight of 10 mild to moderate symptom-type $F_2$ plants (with low virus concentration) produced segregating $F_3$ lines and six of seven severe symptom $F_2$ plants (with high virus concentration) produced all susceptible plants.

The eight segregating $F_3$ lines from the mild to moderate symptom (low virus concentration) $F_2$ plants produced a total of 162 plants with symptoms and 56 symptomless. Chi-square testing for a 3:1 ratio yielded a value of 0.055 ($P = 0.75$–$0.9$). Two of 10 $F_2$ plants in the moderate symptom-lower virus concentration category did not produce segregating $F_3$ lines, whereas one of seven $F_2$ plants in the severe symptom-higher virus concentration category did not produce nonsegregating susceptible $F_3$ lines. This demonstrates that the attempted subgrouping of non-PI 186465-like California Blackeye × PI 186465 $F_2$ plants into heterozygous intermediate and homozygous susceptible plants on the basis of virus concentration and symptoms was generally but not completely accurate.

Segregation of the California Blackeye × PI 186465 and PI 186465 × California Blackeye $F_2$ populations fit a 3:1 ratio for plants with symptoms: symptomless, PI 186465-like plants (Table 4).

**Moderately resistant × extremely resistant.** The Early Pinkeye × PI 186465 $F_1$ was intermediate in both virus concentration and symptom severity to the two parents (Table 1).

Twenty-four of 166 Early Pinkeye × PI 186465 $F_2$ plants were PI 186465-like, with no symptoms and no or negligible virus concentration ($<1$ $\mu$g/g, negative by ELISA) (Table 2). The $F_2$ population included a number of plants with virus concentration higher than the less resistant parent Early Pinkeye (Table 2).

Segregation of the Early Pinkeye × PI 186465 $F_2$ did not acceptably fit a 3:1 ratio for plants more susceptible than PI 186465: PI 186465-like plants (Table 4).

**Resistant × extremely resistant.** The Iron × PI 186465 $F_1$ was similar in virus concentration and symptom severity to the less resistant parent Iron (Table 1).

Forty-two of 175 Iron × PI 186465 $F_2$ plants were PI 186465-like (Table 2). Although there were symptomless plants with virus concentration similar to Iron and symptomless plants that were intermediate in virus concentration to the two parents, PI 186465-like plants were easily distinguished from the others by ELISA. PI 186465-like plants were the only ones that gave a negative ELISA reaction. The Iron × PI 186465 $F_2$ population also included plants with virus concentration higher than Iron.

Segregation in the Iron × PI 186465 $F_2$ fit a 3:1 ratio for plants more susceptible than PI 186465: PI 186465-like plants (Table 4).

## DISCUSSION

The key to the genetic interpretations in this paper is the identification of plants similar to the resistant or more resistant parent in each cross. In crosses between susceptible California Blackeye and each of the resistant lines Early Pinkeye, Iron, and PI 186465, the identification of resistant parent types was carried out by evaluation of both symptoms and virus concentration of $F_2$ plants. In the Early Pinkeye × PI 186465 and Iron × PI 186465 crosses, identification of the more resistant parent-types would, at first glance, seem more difficult, because resistance genes from both parents are segregating in each $F_2$ population. However, the level of resistance in PI 186465 is much greater than in the other

TABLE 4. Chi-square testing of segregation of $F_2$ progeny of cowpea crosses for reaction to southern bean mosaic virus

| Cross[a] | Expected no. genes in more resistant parent[b] | Number of $F_2$ plants: More susceptible than more resistant parent | Number of $F_2$ plants: Equal in resistance to more resistant parent | Expected ratio | Chi-square: Value | Chi-square: Probability |
|---|---|---|---|---|---|---|
| CB × EP | 1 | 122 | 38 | 3:1 | 0.13 | 0.7–0.9 |
| CB × IR | 3 | 171 | 5 | 63:1 | 1.87 | 0.1–0.2 |
| CB × 18 | 1 | 100 | 35 | 3:1 | 0.06 | 0.7–0.9 |
| 18 × CB | 1 | 126 | 39 | 3:1 | 0.16 | 0.5–0.7 |
| EP × 18 | 1 | 142 | 24 | 3:1 | 9.84 | 0.01–0.001 |
| IR × 18 | 1 | 133 | 42 | 3:1 | 0.09 | 0.7–0.9 |

[a] CB = California Blackeye, EP = Early Pinkeye, IR = Iron, and 18 = PI 186465.
[b] Resistance based on restriction of virus concentration: 18 > IR > EP > CB.

two resistant lines, and PI 186465-like plants were easy to identify because they were symptomless and gave negative ELISA reactions (no other group gave negative reactions). Therefore, in each cross evaluated it was possible to separate the $F_2$ into two groups: 1) plants equivalent to the more resistant parent and 2) all other plants. The fact that there were up to six symptom classes represented in some crosses is not really relevant to the genetic interpretation, because plants within the more resistant parent $F_2$ group had only one possible symptom classification. An exception was Iron-like plants in the California Blackeye × Iron $F_2$ population, in which they were either rated as 0 or 0.5. Moreover, the more resistant parent groups were confirmed by their virus concentration.

The different levels of nonnecrotic resistance to SBMV in Early Pinkeye, Iron, and PI 186465, which are clearly distinguishable on the basis of virus concentration and symptoms, also are distinguishable in their pattern of inheritance. Resistance in Early Pinkeye is conferred by a single gene with partial dominance for resistance, similar to the resistance reported for soybean mosaic virus in common bean by Provvidenti et al (15).

Although the data are inconclusive, we believe the resistance in Iron is conditioned by multiple genes (perhaps three or more) with incomplete dominance. It is clear that the control of resistance in Iron is distinct from that in Early Pinkeye and PI 186465. Karchi et al (9) found that three genes conferred resistance to cucumber mosaic virus in a melon cultivar. As with the California Blackeye × Iron cross, they were unable to satisfactorily subclassify susceptible $F_2$ plants from their resistant × susceptible cross due to a wide range of symptoms.

Resistance in PI 186465 is basically monogenic with partial dominance for resistance, but minor genes that affect SBMV concentration and symptoms operate also, when the major gene is not in homozygous form. Minor genes for resistance derived from PI 186465 were apparently segregating along with the major gene, causing fewer California Blackeye × PI 186465 $F_2$ plants to be like the susceptible parent California Blackeye than would be expected for monogenic resistance as well as causing greater variation in the non-PI 186465-like part of the $F_2$ population. Johnson et al (8) found minor genes to be involved in resistance to tobacco etch virus in tobacco along with a single major gene. There were no apparent differences between the California Blackeye × PI 186465 and its reciprocal cross, indicating no cytoplasmic inheritance is involved in resistance from PI 186465.

Support for the single major gene hypothesis for PI 186465 resistance is found in the segregation pattern of the Iron × PI 186465 $F_2$, because one-fourth of the plants had PI 186465-like resistance. However, segregation of the Early Pinkeye × PI 186465 $F_2$ did not fit the expected pattern; there were fewer PI 186465-like plants than would be expected. A possible explanation could be epistasis of the Early Pinkeye gene when in homozygous form over the major PI 186465 gene in homozygous form. Epistasis of one form of virus resistance conferred by a gene over another form conferred by a different gene was reported by Ali (1). Resistance to bean common mosaic virus in bean, conferred by the homozygous recessive gene pair *aa*, was epistatic to resistance conferred by the dominant gene *I*. If the Early Pinkeye gene in homozygous form was epistatic to the major PI 186465 resistance gene in homozygous form, another 1/16 of the Early Pinkeye × PI 186465 $F_2$ would be subtracted from the PI 186465-like category, and a 13:3 ratio would be expected. The observed Early Pinkeye × PI 186465 $F_2$ segregation acceptably fits a 13:3 ratio (chi-square = 2.0, $P = 0.1–0.2$).

Although the data available are insufficient to prove or disprove the hypothesis of such an epistatic relationship, the Early Pinkeye × PI 186465 $F_2$ data indicate that the Early Pinkeye gene and PI 186465 major gene are at different loci. A number of $F_2$ plants had virus concentration much higher than the less resistant parent, Early Pinkeye. If the resistance of the two lines was allelic, the least resistant plants of the $F_2$ would have virus concentration no higher than that of Early Pinkeye.

Determination of virus concentration, rather than symptomatology alone, in individual $F_2$ plants (either by purification of virions or by ELISA) was indispensable for interpretation of some crosses. Identification of the Iron-like class in the $F_2$ population of the California Blackeye × Iron cross required virus concentration evaluation of plants similar in symptom severity to Iron, some of which were found to have virus concentrations much higher than that of Iron. Similarly, identification of the symptomless PI 186465-like class in the Iron × PI 186465 $F_2$ required ELISA differentiation of plants in this group from other symptomless plants with higher virus concentrations.

The resistance gene of Early Pinkeye inhibited SBMV accumulation 95% in the homozygote and 72% in the heterozygote. The *Tm-1* resistance gene in tomato inhibited tobacco mosaic virus accumulation 90–95% in hosts homozygous for *Tm-1* and 65–75% in hosts heterozygous for *Tm-1* (6). The resistance genes of Iron, in homozygous form, allowed five times less SBMV accumulation than Early Pinkeye. The PI 186465 major gene, in homozygous form, allowed at least 100 times less SBMV accumulation than Iron.

Resistance in Clay (necrotic local lesions) allowed SBMV concentration levels intermediate to those of Iron and PI 186465 (11). The nonnecrotic resistance of Early Pinkeye, Iron, and PI 186465 may be preferable to the hypersensitive resistance in Clay, because systemic necrosis may occur in cultivars with hypersensitive resistance to SBMV and other viruses under certain environmental conditions (2,13).

Resistance derived from Early Pinkeye, Iron, and PI 186465 differed in the relationship between virus accumulation and symptoms. In the California Blackeye × Early Pinkeye $F_2$ population, there were three symptom and virus concentration classes that corresponded in symptoms and virus concentration to the parental lines and the $F_1$. In the California Blackeye × Iron $F_2$, there was only one distinct parental symptom-virus concentration class with five plants that bred true; six other similar plants were genetically heterozygous as their $F_3$ lines segregated. Correlation of symptom severity with virus concentration in the entire California Blackeye × Iron $F_2$ population was only fair. In the California Blackeye × PI 186465 $F_2$, there was an easily distinguishable symptomless PI 186465-like group of plants, with extreme resistance to SBMV accumulation, and it bred true for the PI 186465-like reaction. Moreover, there was a good correlation in the population as a whole between symptoms and virus concentration. These results further demonstrate the variability in the relationships between virus concentration and symptom severity (5,6,12,18).

Although resistance to SBMV accumulation in PI 186465 is more complete than in Iron and Early Pinkeye, virus concentration in both Iron and Early Pinkeye is still reduced markedly from that in susceptible lines (11). Earlier work (7) has shown that resistance of both Iron and Early Pinkeye, whether due primarily to reduced virus concentration or to resistance to infection, was sufficient to maintain SBMV field incidence at levels as low as those of PI 186465.

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