Cowpea Stunt: Inheritance Pattern of the Necrotic Synergistic Reaction

G. Pio-Ribeiro, C. W. Kuhn, and B. B. Brantley

Department of Plant Pathology and Plant Genetics, University of Georgia, Athens 30602; and Department of Horticulture, The Georgia Station, Experiment 30212. Present address of senior author: Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Dois Irmãos, 50000 Recife PE., Brazil.

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ABSTRACT

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Cowpea cultivars California Blackeye, Clay, and Iron reacted to an inoculum mixture of cucumber mosaic virus and a Georgia isolate of blackeye cowpea mosaic virus (BlCMV) with severe stunt and necrosis (SSN), severe stunt (SS), and mild mottle, respectively. The F_1 plants of the crosses California Blackeye × Clay, California Blackeye × Iron, and Iron × California Blackeye reacted with severe stunt and intermediate necrosis (SSIN), and F_1 plants of Clay × Iron had an SS reaction. In F_2 populations, the disease reactions segregated in a ratio of 1:2:1 (SSN:SSIN:SS). Thus, the necrotic reaction is controlled by an allele, at a single locus, which exhibits incomplete dominance. This assumption was confirmed by the data from backcrosses and F_3 populations. The resistance of Iron to BlCMV and cowpea stunt was not detected in any plants in the F_1, F_2, F_3, or backcross populations.

Cowpea stunt, which is caused by a synergistic interaction of blackeye cowpea mosaic virus (BlCMV) and cucumber mosaic virus (CMV), was first found in Georgia in 1974 (2). Depending on the cultivar and virus isolate, cowpeas (*Vigna unguiculata* [L.] Walp. subsp. *unguiculata*) react to a mixed inoculum with four major types of symptoms (1): severe stunt with necrosis (SSN), severe stunt (SS), delayed stunt with necrosis, and mild mottle (MM). The MM reaction is caused by CMV only, and the plants are highly resistant to a single infection with BlCMV. Therefore, this study was initiated to determine the inheritance pattern of resistance to BlCMV and consequently to cowpea stunt. The inheritance study also included cowpea cultivars which react with either a necrotic or a non-necrotic synergistic response.

MATERIALS AND METHODS

Cowpea cultivars California Blackeye, Clay, and Iron were used in the following crosses: California Blackeye × Clay, California Blackeye × Iron, Iron × California Blackeye, and Clay × Iron. F_1, F_2, and F_3 populations of these crosses and of several backcrosses were grown in the greenhouse.

Plants of the parental cultivars, of each generation of the crosses, and of the backcrosses were checked for reaction to the Georgia isolate of BlCMV (1), CMV, and BlCMV plus CMV. The virus isolates were maintained in California Blackeye (2). Primary leaves of 9-day-old plants were rubbed with a cheesecloth pad soaked in the inoculm prepared with diseased leaf tissue ground in neutral potassium phosphate buffer (0.01 M) containing 1% Celite® (Celite 545; Fisher Scientific Co., Atlanta, GA 30324). For mixed inoculum, equal volumes of sap (1 g tissue in 4 ml buffer) were mixed just before inoculation, and for the single virus inoculum, the sap was diluted with buffer in the place of the sap containing the second virus. Inoculated plants were maintained in the greenhouse (24–30 C) in 10-cm diameter pots containing a mixture of soil, sand, and vermiculite (2:1:1, v/v) provided with a complete fertilizer. They were observed and evaluated for 4 wk.

Seed for F_2 plants were obtained from noninoculated F_1 plants. Doubly-infected, segregating F_2 plants, with two different degrees of systemic necrosis and with no systemic necrosis, were grown to maturity to provide seed for F_3 plants.

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RESULTS

Reaction of parental cultivars and F_1 plants. Cultivars California Blackeye, Clay, and Iron differed in the type of reaction to single and double inoculations with CMV and BlCMV. The reactions of these cultivars and F_1 populations are summarized in Table 1. The parental reactions to a mixed infection were the same as those reported previously (1). The F_1 plants of the crosses California Blackeye × Clay, California Blackeye × Iron, and Iron × California Blackeye showed severe stunt and intermediate necrosis (SSIN), and F_1 plants of Clay × Iron had an SS reaction. The SSIN reaction was relatively easy to distinguish from SSN. On SSN plants, the first trifoliolate leaves were necrotic and they abscised; in fact, most of the plants died. Infrequently, the second or third trifoliolate leaf had little or no necrosis and the plant survived, although few or no seeds were produced. On SSIN plants, the first and second trifoliolate leaves had some necrosis, but they did not abscise and the plants produced seeds.

TABLE 1. Reaction of parental cultivars and the F_1 generation of four cowpea crosses to double and single mechanical inoculations with cucumber mosaic virus (CMV) and blackeye cowpea mosaic virus (BlCMV)[a]

Parent or cross	Disease reaction[b]		
	CMV + BlCMV	CMV	BlCMV
California Blackeye (CB)	SSN	MM	MM[c]
Clay	SS	MM	MM
Iron	MM[d]	MM	NS
CB × Clay	SSIN	MM	MM[c]
CB × Iron	SSIN	MM	MM[c]
Iron × CB	SSIN	MM	MM[c]
Clay × Iron	SS	MM	MM

[a] Large number of plants of the parental cultivar (>100) and 31–41 F_1 plants of each cross were inoculated.

[b] Letter designations: SSN = severe stunt with necrosis, frequent plant death; SS = severe stunt; MM = mild mottle; SSIN = severe stunt with intermediate necrosis; and NS = no symptoms, but virus recovered by subinoculation infrequently.

[c] Occasional limited necrosis of stems and petioles.

[d] Usually, only CMV could be recovered by subinoculation to CB and Clay.

TABLE 2. Segregation of the F_2 generation of four cowpea crosses and four backcrosses mechanically inoculated with cucumber mosaic virus and blackeye cowpea mosaic virus

Population	Number of plants: Severe stunt, necrosis	Severe stunt, intermediate necrosis	Severe stunt	Expected ratio	Chi-square probability
F_2 generation:					
CB[a] × Clay	92	208	101	1:2:1	0.5–0.75
CB × Iron	99	204	98	1:2:1	0.9–0.95
Iron × CB	102	194	102	1:2:1	0.8–0.90
Clay × Iron	0	0	247	...	...
Backcrosses:					
Iron × F_1 (Iron × CB)	0	93	75	1:1	0.1–0.2
Clay × F_1 (Clay × Iron)	0	0	163	...	...
CB × F_1 (CB × Iron)	74	118	0	1:1	0.001–0.01
CB × F_1 (CB × Clay)	35	54	0	1:1	0.02–0.05

[a]Cultivar California Blackeye.

For single infections, all plants of the three parental cultivars and the F_1 populations reacted to CMV with MM. For BlCMV inoculation, California Blackeye, Clay, and all F_1 plants, regardless of the cross, reacted with mild mosaic; Iron, however, had no symptoms. No segregation occurred in any F_1 population.

Segregation of F_2 and backcross plants. Segregation occurred with the F_2 populations of the crosses California Blackeye × Clay, California Blackeye × Iron, and Iron × California Blackeye, but none was observed with the Clay × Iron cross (Table 2). Two backcrosses involving California Blackeye segregatd with SSN and SSIN reactions, but the ratios did not statistically fit the expected 1:1 ratios (Table 2).

In response to a single infection with CMV, all F_2 and backcross plants (24–28 per cross) showed MM. Similarly, all such plants inoculated with BlCMV (204-226 per cross) reacted with mild mosaic. A few BlCMV-infected plants of crosses of Clay × California Blackeye, California Blackeye × Iron, Iron × California Blackeye, and backcross Iron × F_1 (Iron × California Blackeye) developed limited necrosis of stems and petioles. However, the ratio of segregation could not be determined because the reproducibility of the reaction was very low. This type of reaction to BlCMV was absent in F_2 plants of Clay × Iron and backcross plants of Clay × F_1 (Clay × Iron).

Reaction of F_3 plants. Since the backcross data were inconclusive (Table 2), progeny of F_2 plants showing SSN, SSIN, and SS were checked for reactions to a mixed inoculum of CMV and BlCMV. The results are presented in Table 3. The progeny of F_2 plants with SSN reacted uniformily with SSN, and no segregation was observed in the population originating from F_2 plants with SS. However, the F_3 population obtained from seed of F_2 plants with SSIN segregated, showing plants with SSN, SSIN, and SS.

Resistance in cultivar Iron. The resistance of Iron to BlCMV and cowpea stunt was not detected in any plant in the F_1, F_2, F_3, or backcross populations (Tables 1, 2, 3). To test the possibility of an improper or unsuccessful cross, two additional crosses were made between Iron and California Blackeye. Neither cross produced resistant plants in the F_1 and F_2 populations. Although the parental reaction to BlCMV and cowpea stunt was distinctly different for Iron and Clay (Table 1), progeny of crosses involving the two cultivars all reacted similar to Clay (Tables 1, 2, 3).

Local reaction to CMV. CMV causes necrosis on inoculated leaves of California Blackeye and chlorosis on Clay (1,2). The local necrotic reaction was inherited similarly to the systemic synergistic necrotic reaction caused by a mixed infection of CMV and BlCMV. All F_1 plants had local necrosis that was less intense than that caused in California Blackeye. Segregation for local necrosis and chlorosis occurred in F_2, F_3, and backcross generations. All plants that developed systemic necrosis in response to both viruses also showed the California Blackeye local necrosis reaction in response to CMV. Iron sometimes showed chlorotic and necrotic ringspots in response to CMV. The necrotic ringspot of Iron was not related to systemic necrosis since some plants in F_2, F_3, and backcross generations having Iron as one parent showed this symptom and had no systemic necrosis.

TABLE 3. Reaction of F_3 lines obtained from selected F_2 plants to double mechanical inoculation with cucumber mosaic virus and blackeye cowpea mosaic virus

Line background of crosses	No. of plants[a] (F_2/F_3)	Type of disease reaction[b]
From F_2 plants with SSIN		
Iron × California Blackeye (CB)	1/14	SSN
From F_2 plants with SSIN		
CB × Clay	2/14	SSN, SSIN, SS
CB × Iron	5/51	SSN, SSIN, SS
Iron × CB	2/23	SSN, SSIN, SS
From F_2 plants with SS		
CB × Clay	5/34	SS
CB × Iron	3/42	SS
Clay × Iron	3/32	SS

[a]Letter designations: SSN = severe stunt with necrosis, SSIN = severe stunt with intermediate necrosis, and SS = severe stunt.

DISCUSSION

The synergistic necrotic reaction associated with cowpea stunt is controlled by an allele, designated as *Nv*, at a single locus which exhibits incomplete dominance. The following genotypes for the parental cultivars are in accordance with the data obtained in F_1, F_2, F_3, and backcross generations: California Blackeye—*NvNv* (strong necrosis; frequently death), Clay—*nvnv* (no necrosis), Iron—*nvnv*. The heterozygous condition, *Nvnv* (intermediate necrosis), was observed in all nonparental generations. The latter condition apparently is readily eliminated in breeding programs which develop cultivars. Although seed lots of many cultivars are heterogeneous in their reaction to BlCMV and cowpea stunt, all seed from individual plants produced plants identical to their parents with regard to the synergistic necrotic reaction (1). Similar disease reactions with reciprocal crosses between California Blackeye and Iron demonstrated that maternal inheritance was not involved in the synergistic necrotic reaction.

The original objective of this study was to determine the inheritance pattern of the resistance found in cultivar Iron to the Georgia isolate of BlCMV and cowpea stunt. Iron is rated highly resistant because BlCMV can be recovered only rarely from inoculated plants (1). It was surprising, therefore, to find no resistance in any F_1, F_2, F_3, or backcross plants. If resistance is controlled by several genes, statistically too few plants may have been observed to detect even one plant with resistance. The potential for a susceptible reaction in Iron was demonstrated in a previous study (1) in which the cultivar was susceptible to two isolates of BlCMV and to two isolates of cowpea aphidborne mosaic virus (serologically related to BlCMV). When each of the four isolates was mixed with CMV in the inoculum, the SS synergis-

tic reaction occurred on Iron. This is further evidence that the necrotic reaction is independent of the synergistic stunt phenomenon, and that Iron and Clay have the same genome (*nvnv*) for the necrotic reaction.

The genetic data obtained with single and double inoculations support a relationship between local necrosis on California Blackeye caused by CMV and systemic necrosis caused by CMV plus BlCMV. On the other hand, the systemic necrosis in cowpeas caused by mixed infection of BlCMV and CMV seems to be an extension of the limited necrosis of stems and petioles caused by BlCMV alone in California Blackeye (1,2). This type of reaction to BlCMV segregated in F_2 and backcross generations which had California Blackeye as one parent, but segregation ratios could not be determined because of its low intensity in some plants and its dependence on environment conditions. If the local necrosis caused by CMV alone and the systemic necrosis caused by BlCMV alone have the same genetic basis, the gene for necrosis is fully expressed only in the double infection, and we speculate that both viruses contribute to the SSN reaction.

LITERATURE CITED

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