Four Near-Isogenic Lines of Cotton with Different Genes for Bacterial Blight Resistance

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ABSTRACT

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The development and genetic characterization of four near-isogenic lines (NILs) of cotton (*Gossypium hirsutum*) is described herein. Each line contains a single, but different, gene for resistance to bacterial blight caused by *Xanthomonas campestris* pv. *malvacearum*. The lines were derived using at least six backcrosses to the susceptible recurrent parent

'Acala 44', followed by single plant-progeny row selection for uniformity. The NILs are homozygous for the B_2 , B_4 , B_{In} , or b_7 genes and are designated as AcB_2 , AcB_4 , AcB_{In} , and $Ac b_7$, respectively. In the 'Acala 44' background, B_2 , B_4 , and B_{In} are partially dominant genes; b_7 is partially recessive. Relative strengths of resistance conferred by those genes toward race 1 of the pathogen were $B_4 \sim b_7 > B_{In} \sim B_2$. B_4 , B_{In} , and b_7 each conferred resistance toward *X. campestris* pv. *malvacearum* carrying a single avirulence gene, whereas B_2 was less specific.

Additional keywords: avirulence genes, disease resistance.

Bacterial blight, incited by *Xanthomonas campestris* pv. *malvacearum*, is the only major bacterial disease of cotton (*Gossypium* spp.). In many cotton-growing regions of the world, it reduces lint yield (4) and fiber quality (18). Even though sanitation, quarantine, and various seed treatments have been successfully used as cultural controls, resistant cultivars are generally considered the most effective and dependable method for controlling the disease (3). At least 16 major genes have been identified that confer complete or partial resistance to one or more races of the pathogen (6). Those genes were derived from a number of sources and genetic backgrounds (5). Knight (14,15) transferred B_2 and B_4 plus seven other blight-resistance genes into Sudan Sakel (*Gossypium barbadense*) strains to assess their value for breeding purposes. In the 1950s, Bird (2) transferred B_2 , B_4 , and other genes from Knight's Sakel lines into the upland (*G. hirsutum*) cvs. Empire and Deltapine. The b_7 gene was discovered in 'Stoneville 20' (*G. hirsutum*) by Green and Brinkerhoff (11). The B_{In} gene was found in a *G. hirsutum* cultivar of unknown origin near Indianola, OK, by Green and Brinkerhoff (11). In 1968, Hunter et al. (13) reported they had derived seven permanent and one temporary *G. hirsutum* differential host lines that could distinguish among 15 races of *X. campestris* pv. *malvacearum*. Those lines were selected because they provided reasonably consistent pathogen race differentiation at a number of locations and not because of any genetic relatedness to each other.

A set of near-isogenic lines (NILs) with single, but different, genes for bacterial blight resistance would be useful for studying the physiological and biochemical mechanisms of resistance. To make such studies possible, Hunter and Brinkerhoff (12) began to transfer genes for resistance from several *Gossypium* spp. into *G.*

hirsutum 'Acala 44', a highly susceptible genotype with no known genes for bacterial blight resistance. The crosses were initiated in the greenhouse in the winter of 1959 to 1960, and the first backcrosses to 'Acala 44' were made in the field in 1960 (12). Subsequently, Brinkerhoff made at least three additional backcrosses to 'Acala 44' using one source of resistance to develop the $Ac b_7$ line. The other lines described herein were backcrossed one to three times apiece.

In 1991, De Feyter and Gabriel (8) reported the cloning and identification of six avirulence (*avr*) genes from *X. campestris* pv. *malvacearum*. Each of these, when introduced into a widely virulent strain of the same organism, conferred avirulence toward one or more of Hunter and Brinkerhoff's (12) resistant Acala lines, most of which had only one backcross to the recurrent parent. Later, De Feyter et al. (9) tested interactions between *X. campestris* pv. *malvacearum* strains carrying individual *avr* genes and resistant Acala lines which had been backcrossed two to six times, but which had not yet been subjected to genetic analysis. They indicated that some of the resistance genes (B_4 , b_6 , b_7 , and B_{In}) were specific for single *avr* genes, i.e., a cotton line carrying one of those genes was resistant to only one of the bacterial strains which carried individual *avr* genes. Other resistance genes (B_1 , B_2 , and B_{In3}) appeared to recognize multiple *avr* genes. Nevertheless, a detailed restriction fragment length polymorphism (RFLP) genetic map indicated that B_2 is located in a single region on the D-subgenome chromosome 20 (20).

This report describes the development and genetic characterization of four NILs of cotton carrying bacterial blight-resistance genes B_2 , B_4 , B_{In} , or b_7 . These lines are hereafter designated as AcB_2 , AcB_4 , AcB_{In} , and $Ac b_7$, respectively. When inoculated under Oklahoma field conditions, their levels of resistance to an aggressive strain of *X. campestris* pv. *malvacearum* race 1, which possesses many avirulence genes, were compared with those of the resistant parent lines. Their interactions with De Feyter and Gabriel's (8) isogenic strains of *X. campestris* pv. *malvacearum* carrying individual *avr* genes were tested under growth chamber containment.

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MATERIALS AND METHODS

Genetic stocks and breeding procedures. Hunter and Brinkerhoff's (12) backcross program was suspended by Brinkerhoff's retirement in 1979, but was resumed by Essenberg in 1984. At least five additional backcrosses have now been made to 'Acala 44' (with the last two or more made to Acala 44E) for each of the lines described in this report. Acala 44E is a single plant selection made by Essenberg from Brinkerhoff's original 'Acala 44' parent (5). Selection was based on its general morphology, which was characteristic of 'Acala 44', but with more abundant flowering.

Two cotton nurseries were utilized during the development of these NILs—a summer nursery near Perkins, OK, on a Teller loam soil (a fine-loamy, mixed, active, thermic Udic Argiustoll) and a winter nursery near Tecmán, Mexico, on a La Escondida silty clay loam soil (a fine, smectitic, mixed, isohyperthermic Calcic Argiustoll). Disease screenings as well as the backcrosses to 'Acala 44' or Acala 44E were made at Perkins. The winter nursery was used to increase seed stocks of parents and to advance F_1 populations of the backcrosses to the F_2 by self-pollination. In addition, the crosses to verify the inheritance of the genes in the NILs were made in Mexico.

After each backcross, the F_2 populations were screened before flowering by spray inoculation (described below) at Perkins, susceptible plants were rogued, and those remaining were used as parents for the next backcross. After each line had received at least six backcrosses to 'Acala 44' or Acala 44E, selected plants were self-pollinated and individually harvested. The following summer, in each line, four to seven progeny rows (which each traced back to a single plant) were planted, spray-inoculated, and screened. One progeny row with uniform resistance was selected to be the final NIL for that gene. Each progeny row had at least 16 uniformly resistant plants and had none with the more susceptible grades of 2.3 or 4.0 (explained below). Crosses were then made to measure the degree of dominance for each gene as well as to test the hypothesis that the resistance was inherited as a single locus.

Disease resistance screening was conducted in the field or in growth chambers, depending upon the nature of the testing being done and the strains being utilized.

Pathogen isolates. Initial screening of the NILs was accomplished with *X. campestris* pv. *malvacearum* race 1 isolates from the collection maintained by Brinkerhoff (6). Subsequent work

utilized the strains listed in Table 1. The crosses to determine degree of dominance and inheritance were all screened in the field with strain 3631 (17). The 1003 strains were in a wild-type African strain background with a wider host range than New World strains (8). Therefore, they were used only in biocontainment environments.

All of the strains except Brinkerhoff's race 1 and strain 3631 were provided by D. W. Gabriel. Plasmid pUFR047 was derived from plasmid pUFR042 by replacing the gene for kanamycin resistance with a gene for ampicillin resistance (9), a change that had no detected effect on pathogenicity or host range of *X. campestris* pv. *malvacearum*. Although strain 3631 was derived from a field isolate that exhibited race 3 phenotype [virulent in *G. hirsutum* 1-10B, which possesses the bacterial blight-resistance gene B_{1n} (6)] in 1978 (10,17), in the work described here it exhibited race 1 phenotype [avirulent in all the differential lines carrying resistance genes (6)]. Cultures were maintained frozen at -70°C in 14% (vol/vol) glycerol in Bacto nutrient broth (Difco Laboratories, Detroit, MI).

Resistance screening in the field. Segregating F_2 populations from each of the NILs were planted at Perkins to identify those plants carrying the desired resistance genes. In subsequent years, parental, F_1 , F_2 , and backcross populations were also included. Progeny rows were spaced 100 cm apart and were 10.5 m long. Plants in each row were thinned to approximately 30 cm prior to inoculation.

Inoculum was prepared by culturing *X. campestris* pv. *malvacearum* 3631 (race 1) in nutrient broth at 30°C with shaking (≈ 150 rpm) to late logarithmically growing phase ($\approx 10^9$ CFU ml^{-1}). Cultures were taken to the field, kept out of direct sunlight, and diluted to 1 to 5×10^6 CFU ml^{-1} in unchlorinated well water just prior to use. Plants were inoculated at approximately the six to seven true-leaf growth stage by spraying the bacterial suspension onto the abaxial surface of several leaves on each plant. Spraying was done with a single-nozzle, orchard-type spray gun operating at a pressure of approximately 1.0 to 1.4×10^6 Pa. Up to 50% of the total leaf area of each plant was infiltrated. Viability and pathogenicity of each tank of inoculum was checked by syringe inoculation into susceptible Acala 44E just before the tank was emptied, followed by examination of those inoculated areas for disease symptoms at 1 to 2 weeks.

Plants were classified for disease reactions beginning approximately 14 days after inoculation. Scores were recorded for each plant based on the scale described by Brinkerhoff et al. (7). The disease grading scale is based on the size and shape of visible lesions: 0 = immune, no visible lesions; 1 = resistant, dry, small, round to angular lesions; and 4 = susceptible, large (1 to 2 mm), water-soaked, angular lesions that turn black on drying plus up to 0.5 cm-long water-soaked vein lesions. Four additional grades were used to describe mesothetic reactions: 0.1 and 0.2 = predominantly immune with a few pinpoint (0.1) to small, round (0.2) lesions; 1.2 = resistant, predominantly small, angular lesions, but with small, round lesions also present; and 2.3 = intermediate, predominantly small, angular lesions but with large, water-soaked, angular lesions also present. Susceptible and intermediate plants were removed, and only those plants with resistant and immune phenotypes were used for backcrosses.

To facilitate the determination of a mean bacterial blight grade to estimate degree and direction of dominance, a disease index was established for plants graded in the field. That index corresponded to the field grades (in parentheses) as follows: 0 (0.0), 1 (0.1), 2 (0.2), 3 (1.0), 4 (1.2), 5 (2.3), and 6 (4.0). The presumption of such an index is of equidistance between field grades. It assumes that the genetic distance (or difference) between 0.0 and 0.1 is equivalent to that between 2.3 and 4.0, etc. Other indexes could easily have been derived, but they would have the same shortcoming in that there is not an objective way to choose "the best" one. Segregation data from the field were analyzed using

TABLE 1. Strains of *Xanthomonas campestris* pv. *malvacearum* used in this study

Strain	Relevant genotype or phenotype	Reference
Field isolates	Race 1, widely avirulent	Brinkerhoff (6)
3631	Race 1, widely avirulent, Sm^{Rz}	Pierce et al. (17)
H	Race 1, widely avirulent	De Feyter and Gabriel (8)
1003/pUFR042	Widely virulent, plasmid contains no insert	De Feyter and Gabriel (8)
1003/pUFR123	Contains pUFR042 carrying an 11.6 kb <i>Eco</i> R1 fragment containing <i>avrB</i> ₄	De Feyter and Gabriel (unpublished)
1003/pUFR127	Contains pUFR042 carrying <i>avrB</i> ₆	De Feyter and Gabriel (8)
1003/pUFR163	Contains pUFR042 carrying <i>avrB</i> ₇	De Feyter et al. (9)
1003/pUFR156	Contains pUFR042 carrying <i>avrB</i> _{1n}	De Feyter et al. (9)
1003/pUFR142	Contains pUFR047 carrying <i>avrB</i> ₁₀₁	De Feyter et al. (9)
1003/pUFR157	Contains pUFR042 carrying <i>avrB</i> ₁₀₂	De Feyter et al. (9)

^z Streptomycin resistant.

chi-square procedures (19). Plants grading 2.3 and 4.0 were classified as “susceptible”, while those grading 1.2 or less were classified as “resistant” (7), unless otherwise noted. Mid-parent values and degrees of dominance were calculated, where possible, using standard definitions (1).

Tests of host/race specificity. Cotton plants were grown in a peat/vermiculite mixture (Jiffy Mix Plus, Jiffy Products of America, Batavia, IL) in growth chambers with a 14-h light/10-h dark cycle. Maximum air temperature during the photoperiod was 30°C; minimum temperature during the dark period was 19°C. Plants were fertilized weekly with a mineral mixture (Miracle-Gro All Purpose Plant Food, Scotts Miracle-Gro Products, Inc., Port Washington, NY). Each of the NILs was tested for host/race specificity. These tests included 14 to 20 plants of the NIL plus two plants each of Acala 44E and line Im216 (7) to check the susceptible and immune extremes, respectively, of the disease grading scale. Inoculation was done 6 to 15 weeks after planting, using the youngest, fully expanded leaves. Transparent plastic bags were placed over the leaves 1 to 2 h before inoculation to encourage stomatal opening in response to the increased humidity. Inocula of *X. campestris* pv. *malvacearum* H (race 1) and the seven 1003 strains (Table 1) were prepared by diluting late logarithmic phase nutrient broth cultures in sterile, saturated CaCO₃ solution to $\approx 5 \times 10^6$ CFU ml⁻¹. Inoculation was accomplished with needleless syringes through open stomata on the abaxial leaf surface. A single leaf of each plant was inoculated in four places with each of the strains being tested.

Disease grades of plants in the growth chamber were evaluated every 2 days, beginning 7 days postinoculation. The grades reported here are those recorded on the day for each strain when the highest average grade was attained, usually 11 or 13 days post-inoculation. The disease grade scale was based on the sizes of the macroscopically visible water-soaked areas: 0 = no water-soaking; 1 = pinpoint-sized dots; 2 = small, round speckles (≈ 0.3 mm); 3 = merged angular patches; and 4 = confluent areas. Intermediate grades between each of the established grades, e.g., 1.3, 2.7, and 3.3, were sometimes recorded.

For each NIL, the growth chamber data consisted of the disease grades attained by each of eight strains of *X. campestris* pv. *malvacearum* in a set of 14 to 20 plants. The grades attained by any two strains on a given plant were considered paired data. The paired sample *t* test was used to determine whether responses of the NIL to those two strains differed at the 0.05 probability level.

RESULTS

The segregation data support the conclusion that the NILs for *B₂*, *B₄*, and *B_{ln}* each possess a single gene for resistance (Table 2). Those genes were dominant to some degree, confirming the original reports describing them (11,14,16). The original model that defined “resistance” as grades 0.0 through 1.2 inclusive and defined “susceptible” as grades 2.3 and 4.0 did not work as well for the NIL containing *b₇*. When the susceptible class was expanded to include plants with grades of 1.2, the line was not rejected by

TABLE 2. Bacterial blight reactions of parental, F₁, backcross, and F₂ populations inoculated in the field with race 1 of *Xanthomonas campestris* pv. *malvacearum*

Near-isogenic line ^u	Population	Hypothesis tested	Number of plants		χ^2	<i>P</i>
			R ^v	S ^w		
<i>AcB₂</i> ^x	Acala 44E	(0:1)	0	192 ^y		
	<i>AcB₂</i>	(1:0)	30	0		
	Acala 44E × (Acala 44E × <i>AcB₂</i>)	(1:1)	70	55	1.57	0.20-0.30
	<i>AcB₂</i> × (Acala 44E × <i>AcB₂</i>)	(1:0)	95	0		
	(Acala 44E × <i>AcB₂</i>) F ₂	(3:1)	231	67	0.88	0.30-0.50
<i>AcB₄</i>	Acala 44E	(0:1)	0	192 ^y		
	<i>AcB₄</i>	(1:0)	31	0		
	(Acala 44E × <i>AcB₄</i>) F ₁	(1:0)	14	0		
	Acala 44E × (Acala 44E × <i>AcB₄</i>)	(1:1)	12	14	0.04	0.70-0.90
	<i>AcB₄</i> × (Acala 44E × <i>AcB₄</i>)	(1:0)	31	0		
<i>AcB_{ln}</i>	(Acala 44E × <i>AcB₄</i>) F ₂	(3:1)	188	59	0.11	0.70-0.90
	Acala 44E	(0:1)	0	50		
	<i>AcB_{ln}</i>	(1:0)	46	0		
	(Acala 44E × <i>AcB_{ln}</i>) F ₁	(1:0)	48	0		
	Acala 44E × (Acala 44E × <i>AcB_{ln}</i>)	(1:1)	48	55	0.35	0.50-0.70
<i>AcB₇</i>	<i>AcB_{ln}</i> × (Acala 44E × <i>AcB_{ln}</i>)	(1:0)	113	0		
	(Acala 44E × <i>AcB_{ln}</i>) F ₂	(3:1)	234	71	0.39	0.50-0.70
	Acala 44E	(0:1)	0	192 ^y		
	<i>AcB₇</i>	(1:0)	46	0		
	(Acala 44E × <i>AcB₇</i>) F ₁	(0:1)	5	16		
<i>AcB₇</i> alternate ^z	Acala 44E × (Acala 44E × <i>AcB₇</i>)	(0:1)	50	68		
	<i>AcB₇</i> × (Acala 44E × <i>AcB₇</i>)	(1:1)	57	36	4.30	0.01-0.05
	(Acala 44E × <i>AcB₇</i>) F ₂	(1:3)	111	131	55.1	<0.001
	Acala 44E	(0:1)	0	192 ^y		
	<i>AcB₇</i>	(1:0)	46	0		
	(Acala 44E × <i>AcB₇</i>) F ₁	(0:1)	2	19		
	Acala 44E × (Acala 44E × <i>AcB₇</i>)	(0:1)	10	108		
	<i>AcB₇</i> × (Acala 44E × <i>AcB₇</i>)	(1:1)	50	43	0.39	0.50-0.70
	(Acala 44E × <i>AcB₇</i>) F ₂	(1:3)	73	169	3.17	0.05-0.10

^u Near-isogenic populations were tested in the following years: *AcB₂* (1994), *AcB₄* (1994), *AcB_{ln}* (1991), and *AcB₇* (1994).

^v Resistant plants are defined as grades 0.0 through 1.2 unless otherwise noted.

^w Susceptible plants are defined as grades 2.3 and 4.0 unless otherwise noted.

^x No F₁ plants were available for this line in 1994.

^y The total number of Acala 44E plants included in all test plots in 1994.

^z The alternate *AcB₇* proposal uses the same data as the original *AcB₇* proposal above it. In this case, resistant plants are defined as grades 0.0 through 1.0; susceptible plants are defined as grades 1.2 through 4.0.

TABLE 3. Mean disease index, closest grade, and degree of dominance in field data for near-isogenic lines

Near-isogenic line ^v	Population	Number of plants	Mean disease index ^w	Closest grade ^w	Degree of dominance ^x
AcB ₂	Acala 44E	192 ^y	5.8	4.0	... ^z
	AcB ₂	30	3.3	1.0	
	(Acala 44E × AcB ₂) F ₁	0 ^z	... ^z	... ^z	
	Mid-parent	222	4.5	2.3	
AcB ₄	Acala 44E	192 ^y	5.8	4.0	-0.46
	AcB ₄	31	1.5	0.1	
	(Acala 44E × AcB ₄) F ₁	14	2.6	1.0	
	Mid-parent	223	3.6	1.2	
AcB _{1n}	Acala 44E	50	6.0	4.0	-0.83
	AcB _{1n}	46	3.2	1.0	
	(Acala 44E × AcB _{1n}) F ₁	48	3.5	1.0	
	Mid-parent	96	4.6	2.3	
Acb ₇	Acala 44E	192 ^y	5.8	4.0	0.51
	Acb ₇	46	1.7	0.2	
	(Acala 44E × Acb ₇) F ₁	21	4.8	2.3	
	Mid-parent	238	3.8	1.2	

^v Near-isogenic populations were tested with race 1 of *Xanthomonas campestris* pv. *malvacearum* in the following years: AcB₂ (1994), AcB₄ (1994), AcB_{1n} (1991), and Acb₇ (1994).

^w Disease indexes (and corresponding grades) were as follows: 0 (0.0), 1 (0.1), 2 (0.2), 3 (1.0), 4 (1.2), 5 (2.3), and 6 (4.0). Blight grades ranged from 0.0 (immune) through 4.0 (susceptible).

^x Degree of dominance calculations were based on disease index values. Negative estimates were toward lower (more resistant) grades; positive estimates were toward higher (more susceptible) grades. The resistant parent is scored as -1.00, whereas the susceptible parent is scored as 1.00.

^y The total number of Acala 44E plants included in all test plots in 1994.

^z No F₁ plants were available for this line in 1994.

TABLE 4. Comparisons of bacterial blight reactions in the field for host differential series and near-isogenic lines when tested with race 1 of *Xanthomonas campestris* pv. *malvacearum*

Line ^v	Designation	Gene	Number of plants	Mean disease index ^{w,x}	Closest grade ^w
'Acala 44'	Differential	None ^y	37	5.8 a	4.0
Acala 44E	Susceptible recurrent parent	None ^y	82	5.9 a	4.0
Mebane B-1	Differential	B ₂	28	4.4 a	1.0-1.2 ^x
AcB ₂	Near-isogenic line	B ₂	103	4.0 a	1.0-1.2 ^x
AcB ₄ ^z	Near-isogenic line	B ₄	186	2.1	0.2
1-10B	Differential	B _{1n}	12	3.1 a	1.0
AcB _{1n}	Near-isogenic line	B _{1n}	172	3.7 b	1.2
'Stoneville 20'	Differential	b ₇	44	1.3 a	0.1
Acb ₇	Near-isogenic line	b ₇	298	1.6 b	0.2

^v Near-isogenic and differential populations were tested in the following years: 'Acala 44' and Acala 44E (1993 and 1996), Mebane B-1 and AcB₂ (1994, 1995, and 1996), AcB₄ (1994, 1995, and 1996), 1-10B and AcB_{1n} (1996), and 'Stoneville 20' and Acb₇ (1994, 1995, and 1996).

^w Disease indexes (and corresponding grades) were as follows: 0 (0.0), 1 (0.1), 2 (0.2), 3 (1.0), 4 (1.2), 5 (2.3), and 6 (4.0). Blight grades ranged from 0.0 (immune) through 4.0 (susceptible).

^x Within each pair of lines, mean values followed by the same letter were not significantly different according to an unpaired sample *t* test at the 0.05 probability level.

^y These entries appear to have some weak, segregating resistance.

^z There is no matching differential series host for AcB₄.

the chi-square test indicating a single gene. The segregation pattern for this gene indicated that it was at least partially recessive, confirming its original report (11).

The degree and direction of dominance for genes B₄ and B_{1n} were -0.46 and -0.83 toward the lower (more resistant) grades, respectively; for gene b₇, they were 0.51 toward the higher (more susceptible) grades (Table 3). Degree and direction of dominance were not calculated for B₂ because of the lack of F₁ data in the test year reported.

Comparisons were made between the reported disease grades for Brinkerhoff's differential series (6,12,13) and our NILs AcB₂, AcB_{1n}, and Acb₇ (Table 4). There is no matching line for AcB₄ in the differential series. In each case, the differential based on "mean disease index" exhibited equal or slightly greater disease resistance than our NIL (Table 4). The differentials containing B_{1n} and b₇ were significantly more resistant than their corresponding NILs; AcB₂ equaled its differential, as did Acala 44E.

The specificities of the bacterial blight-resistance genes toward six *avr* genes of *X. campestris* pv. *malvacearum* were tested

(Table 5). Fourteen to 20 plants of each NIL, grown in a growth chamber, were inoculated with the isogenic strains of *X. campestris* pv. *malvacearum* developed by De Feyter and Gabriel (8) and De Feyter et al. (9) which carry individual *avr* genes. The mean quantitative disease grades attained for all combinations of NILs and *avr* genes are shown in Table 5. The qualitative patterns of resistance and susceptibility deduced from the statistical relationships among those means are also shown in Table 5. AcB₄ gave a resistant reaction only to the individual gene *avrB₄*, as did AcB_{1n} to *avrB_{1n}* and Acb₇ to *avrb₇*. However, AcB₂ exhibited resistant reactions to genes *avrB₄* and *avrB₁₀₁*; intermediate reactions to *avrb₆*, *avrb₇*, and *avrB₁₀₂*; and a susceptible response to *avrB_{1n}*.

DISCUSSION

Each of the four NILs was derived using at least six backcrosses to 'Acala 44' or Acala 44E. Therefore, on the average, each NIL was 99.2% 'Acala 44' and/or Acala 44E (1). With a high

TABLE 5. Host/race specificities in growth chambers: Infection phenotypes of isogenic races in the near-isogenic lines

<i>Xanthomonas campestris</i> pv. <i>malvacearum</i> strain	Cotton lines						
	Acala 44E	AcB ₂	AcB ₄ Test 1 ^v	AcB ₄ Test 2 ^v	AcB _{ln}	AcB ₇	Im216
Quantitative disease grades ^{w,x,y}							
1003/pUFR042 (vector)	3.23 a	3.15 a	2.84 a	3.84 a	2.64 a	2.79 a	0.00 b
1003/pUFR123 (<i>avrB₄</i>)	3.28 a	0.57 b	0.00 b	...	2.80 a	2.99 a	0.00 b
1003/pUFR127 (<i>avrB₆</i>)	2.60 a	1.29 ab	... ^y	3.97 a	3.14 a	2.53 a	0.00 b
1003/pUFR163 (<i>avrB₇</i>)	3.05 a	2.25 ab	2.95 a	...	3.39 a	0.00 b	0.00 b
1003/pUFR156 (<i>avrB_{ln}</i>)	3.27 a	2.75 a	2.98 a	...	0.12 b	3.01 a	0.00 b
1003/pUFR142 (<i>avrB₁₀₁</i>)	3.32 a	0.18 b	2.81 a	...	2.36 a	3.00 a	0.00 b
1003/pUFR157 (<i>avrB₁₀₂</i>)	3.17 a	1.26 ab	2.44 a	...	2.28 a	2.89 a	0.00 b
H (all 6 <i>avr</i> genes)	3.67 a	0.09 b	0.00 b	0.00 b	0.27 b	0.00 b	0.00 b
Qualitative patterns of specificity ^z							
1003/pUFR042 (vector)	+	+	+	+	+	+	—
1003/pUFR123 (<i>avrB₄</i>)	+	—	—	...	+	+	—
1003/pUFR127 (<i>avrB₆</i>)	+	+/-	...	+	+	+	—
1003/pUFR163 (<i>avrB₇</i>)	+	+/-	+	...	+	—	—
1003/pUFR156 (<i>avrB_{ln}</i>)	+	+	+	...	—	+	—
1003/pUFR142 (<i>avrB₁₀₁</i>)	+	—	+	...	+	+	—
1003/pUFR157 (<i>avrB₁₀₂</i>)	+	+/-	+	...	+	+	—
H (all 6 <i>avr</i> genes)	+	—	—	—	—	—	—

^v During test 1, the culture of strain 1003/pUFR127 (*avrB₆*) proved to be contaminated, which required the retesting of AcB₄ in test 2.

^w Disease grade scale was as follows: 0 = no water-soaking; 1 = pinpoint-sized dots; 2 = small, round speckles (≈0.3 mm); 3 = merged angular patches; 4 = confluent areas. Intermediate grades between each of the established grades, e.g., 1.3, 2.7, and 3.3, were sometimes recorded.

^x Except for Acala 44E and Im216 (an immune line), each value is the mean for 14 to 20 plants of an individual near-isogenic line (NIL) grown and tested together. The Acala 44E and Im216 values are the overall means obtained from two plants of each that were grown and tested with each NIL.

^y Within each column, mean values followed by the same letter were not significantly different according to a paired sample *t* test at the 0.05 probability level.

^z The quantitative data determined the qualitative patterns.

degree of probability, differences between NILs can be attributed to their differences in genes for blight resistance. The NILs also underwent single plant—progeny row selection, which increased within-line uniformity for the trait selected (1). Each NIL appears to be homozygous for one of the blight-resistance genes *B₂*, *B₄*, *B_{ln}*, or *b₇*. Because resistance was retained through backcrosses in which the female parent was the susceptible recurrent parent, all are genes located in the cell nucleus, not in the cytoplasm. In the 'Acala 44'/Acala 44E background, *B₂*, *B₄*, and *B_{ln}* were partially dominant; *b₇* was partially recessive.

The slightly higher level of resistance found in the differential lines with genes *B_{ln}* and *b₇* than in these backcross lines is consistent with the hypothesis that those differentials contain polygenes that enhance resistance to race 1. In the backcross method, if the susceptible parent is used as the recurrent parent (as was done here), polygenes (with their small effects) tend to be lost with each cross (7). The backcross program followed herein appears to have had that result for those two NILs. Slight differences in closest field grades were noted for AcB₄ and AcB_{ln} in Table 4 (1996) versus Table 3 (other years). These could have resulted from differences in environment and/or graders between those years.

The four bacterial blight-resistance genes differed in strength of resistance that they conferred toward *X. campestris* pv. *malvacearum* race 1 (as exemplified by strain H). Their strengths appeared to be somewhat dependent on environment, differing slightly from year to year in the field (Table 3 versus Table 4). In the growth chambers, all NILs were strongly resistant to race 1 (strain H). Approximately the same order of relative strength was observed in the field (Table 4) as in growth chambers (Table 5): *B₄*~*b₇*>*B_{ln}*~*B₂*.

The testing of each NIL with individual isogenic races (Table 5) showed the *avr* gene specificity of each resistance gene. This race collection includes all six of the *avr* genes isolated by De Feyter and Gabriel (8) and De Feyter et al. (9). For the resistance genes common between our study and theirs, our pattern of host/race specificities is essentially the same as the one they observed (8), using the 'Acala 44' lines at an earlier stage in the backcross program. *B₄*, *B_{ln}*, and *b₇* were each specific for one of the *avr* genes, thus they exhibited gene-for-gene relationships. The resistance in

B₂ was less specific (Table 5). Genetically, *B₂* segregated as a single gene; but in reality, it could be two or more closely linked genes. Another intriguing possibility is that it could be a single gene exhibiting the horizontal characteristics of low specificity and low levels of resistance.

These single-gene NILs should prove useful to breeders interested in mapping or cloning the *B* genes and to other researchers interested in studying the effects of genes which confer resistance to bacterial blight (without the complication of differing genetic backgrounds). The lines AcB₄, AcB_{ln}, and Acb₇ are currently being used in our program to construct a gene pyramid with all possible combinations of one, two, or three genes. That pyramid will be used to study the changes when multiple versus single resistance genes are present. Limited quantities of seed for each of the NILs can be obtained upon written request to Margaret Essenberg.

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