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INHERITANCE OF AN INDUCED MUTATION FOR BACTERIAL BLIGHT RESISTANCE IN COTTON[1]

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ABSTRACT

An induced mutation for an intermediate level of resistance to bacterial blight [*Xanthomonas malvacearum* (E. F. Sm.) Dows.] in cotton (*Gossypium hirsutum* L.) was obtained after irradiating seed of a blight-susceptible cultivar, 'Westburn 70', with fission neutrons. Inheritance studies indicated that the resistance was due to a single dominant gene. The mutant was not identical to B_2, B_4, B_5, b_7, or B_N. Tests of homology with 12 other known bacterial blight resistance genes have not yet been conducted.

Additional index words: ***Xanthomonas malvacearum*** **(E. F. Sm.) Dows.,** ***Gossypium hirsutum*** **L., Mutation breeding, Fission neutrons.**

RECENT reports from the USSR indicate that ionizing radiations were useful in breeding for resistance to fusarium [*Fusarium oxysporum* Schlecht. f. *vasinfectum* (Atk.) Snyd. and Hans.] wilt (7) and verticillium (*Verticillium dahliae* Kleb.) wilt (8) of cotton (*Gossypium* spp.) and that chemical mutagens were also helpful in obtaining resistance to fusarium wilt (6) in *G. barbadense* L. This report describes the inheritance of a mutation for resistance to bacterial blight [*Xanthomonas malvacearum* (E. F. Sm.) Dows.] discovered after the irradiation of seed of a susceptible cotton (*G. hirsutum* L.) cultivar. To the authors' knowledge, this is the first induced mutation obtained for bacterial-blight resistance in cotton.

MATERIALS AND METHODS

Foundation seed of the bacterial blight-susceptible, upland cotton cultivar 'Westburn 70' (9) were irradiated at Oak Ridge, Tenn., by M. J. Constantin of the University of Tennessee—Atomic Energy Commission Agricultural Research Laboratory. The seed lot discussed herein (sample code no. UT-72-474 Okla. 6) received 1,375 rads of fission neutrons in April, 1972. At the time of treatment, the seed had a moisture content of 8%. The M_1 seed were planted in 1972 near Altus, Okla., at a considerable distance from other cotton and with several different crops interspersed between them. Cross-pollination data at Altus were unavailable, 0.3%, and 3.0% for 1972, 1973, and 1974, respectively (Verhalen, unpublished data). Crossing between plants in adjacent fields was considered unlikely. In the rare event that it did occur, the cultivar commonly grown in the area is 'Deltapine 16', which is itself susceptible to blight. Open-pollinated plants were grown in 1973 (M_2) as a bulk under isolation and again in 1974 (M_3) under isolation as progeny rows at the same location. Selections among individual M_2 plants were based on characters other than blight resistance.

Seventy-five M_3 progeny rows were inoculated at the four-to-six leaf stage with a cell suspension (1×10^6 cells/ml) of the predominant race (race 1) of *X. malvacearum*. For the inoculations, a single nozzle, orchard-type spray gun was operated at approximately 10.5 kg/cm² (150 psi) from a quad pump driven by the power take-off of a farm tractor. Progeny rows were 1.0 m apart and 7.6 m long, and plants within rows were about 15 cm apart.

Two weeks after inoculation, the progeny rows were graded for bacterial blight symptoms; and all were found fully susceptible except for three plants in one row. The level of resistance exhibited by those three plants was relatively low (Fig. 1) and was similar for each plant. The resistant plants were self pollinated; and one progeny row of each was grown in a nursery at Stillwater, Okla., in 1975 where they were inoculated as previously described. The culture of race 1 of the bacterium used in 1975 proved to be attenuated, as fully susceptible 'Acala 44' checks were only mildly diseased. Single-row progenies from each of the three resistant plants segregated for resistant, intermediate, and mildly susceptible disease reactions. As the plant populations were small and the mildly susceptible reactions atypical, segregation ratios were not recorded.

Three resistant plants (#1, #2, and #12) in one of the segregating progenies (designated as Westburn 70-M2) were used as pollen parents in crosses with the blight-susceptible cultivar, Acala 44, at Stillwater, Okla. The F_1 generations were grown near Iguala, Mexico, during the 1975-76 winter season; and selfed seed from those F_1 plants were harvested individually. During the same season, backcrosses were made to progeny of the susceptible parent and to progeny of one of the resistant parents (plant #1). Seed of the three original selections from Altus in 1974 were grown in both Stillwater in 1975 and in Mexico in 1975-76. At Stillwater, the phenotypes of the M_4 progenies were normal; in Mexico, all exhibited severe dwarfing, later flowering, and reduced fertility. Only one plant in a progeny from plant #2 matured selfed seed in Mexico. The M_5 progenies from the resistant plant #1 selected in Stillwater appeared relatively normal in Mexico. These progeny were used to make the backcrosses. The F_1 populations in Mexico were fruitful and normal in appearance as were the non-irradiated parental line and other materials sent to Mexico that season.

The parental, F_2, and backcross populations were grown at Perkins, Okla., in a planting made 7 June 1976. Plants within progeny rows were thinned to approximately 15 cm, and the plants were inoculated (as previously described) on 7 July with a fully virulent culture of race 1 of *X. malvacearum*. Symptoms of bacterial blight were uniform and severe on susceptible plants throughout the test; disease expression was favored by 3.5 cm of rain on 16 July. Plants were classified for their disease reactions on 20 and 21 July. Only two classes were distinguished, i.e., intermediate (or moderate) resistance and susceptible (Fig. 1). The phenotype of the resistant parental lines was reasonably normal, including the progeny derived from the single plant that had produced selfed seed in Mexico. No dwarf, late flowering, or sterile plants were noted in the F_2's at Perkins.

Small populations of two of the F_1's (together with resistant and susceptible parents) were grown in the greenhouse at Stillwater, inoculated, and classified for their disease reactions during the winter of 1976-77.

Genetic relationships between the Westburn 70-M2 mutant and tester lines homozygous for single genes conferring blight resistance were determined by comparing their disease reactions after syringe inoculating (3) single leaves with races 1, 2, 4, and "ACCO 10" of *X. malvacearum*. "ACCO 10" is currently being described as race 18 (L. S. Bird, personal communication). Three additional isolates of *X. malvacearum*, not identified as to race, were also included in these comparisons. Different genes would exhibit different responses to at least one race tested, provided a critical race was used in the screening. The same response may or may not indicate the same gene. Only a genetic study with the appropriate generations and races can make that determination.

Comparisons were also made of the development of lesions from spontaneous mutants (4) from race 1 on the tester lines and the mutant line. In environments which especially favor bacterial blight (as in 1976 at Perkins), many (but not all) single-gene, blight-resistant lines develop a few water-soaked sus-

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ceptible-type lesions after the initial resistant reaction has been expressed. Isolates from those water-soaked lesions typically react as new races that are fully pathogenic to the hosts from which they were obtained (3, 4). Interpretation of differences or similarities of response to these mutants is the same as for the original races as described above.

RESULTS AND DISCUSSION

The segregation data (Table 1) indicate that the intermediate level of blight resistance exhibited by Westburn 70-M2 is the result of a mutation at a single locus and that the mutant allele is expressed as a complete dominant. However, inoculation of the segregating parental lines with the attenuated inoculum (inadvertantly used in 1975) produced resistant, intermediate, and susceptible classes of plants indicating partial dominance *in special circumstances*. The low-virulence inoculum in 1975 did have a beneficial effect because it enabled us to select homozygous resistant parents for use in crosses. Only two classes (intermediate and susceptible) could be distinguished with the fully virulent inoculum used in 1976.

Although the growth pattern of the M_4 blight-resistant mutant was normal in Oklahoma, its phenotype was greatly affected by day length and possibly other factors in Mexico. Thus, it appears relatively certain that some mutation damage resulted from irradiation of the original seed because non-irradiated lines were normal under the environments of both

Table 1. Bacterial blight reactions of parental, F_1, F_2, and backcross populations inoculated with race 1 of *X. malvacearum*.

Population	Hypothesis tested	No. of plants: Resistant	No. of plants: Susceptible	χ^2	P
Westburn 70-M2#1	(1:0)	13	0	--	--
Westburn 70-M2#2	(1:0)	33	0	--	--
Westburn 70-M2#12	(1:0)	29	0	--	--
Acala 44	(0:1)	0	248	--	--
F_1 Ac44 × Wb70-M2#1	(1:0)	4	0	--	--
F_1 Ac44 × Wb70-M2#12	(1:0)	16	0	--	--
F_2 Ac44 × Wb70-M2#1	(3:1)	314	109	0.079	0.70-0.90
F_2 Ac44 × Wb70-M2#2	(3:1)	425	148	0.189	0.50-0.70
F_2 Ac44 × Wb70-M2#12	(3:1)	400	148	1.073	0.20-0.30
Pooled F_2 (Homogeneity)	(3:1)	1,139	405	0.277	0.70-0.90
Bc_1 Ac44 × (Ac44 × Wb70-M2#1)	(1:1)	65	62	0.031	0.70-0.90
Bc_1 Wb70-M2#1 × (Ac44 × Wb70-M2#1)	(1:0)	145	0	--	--

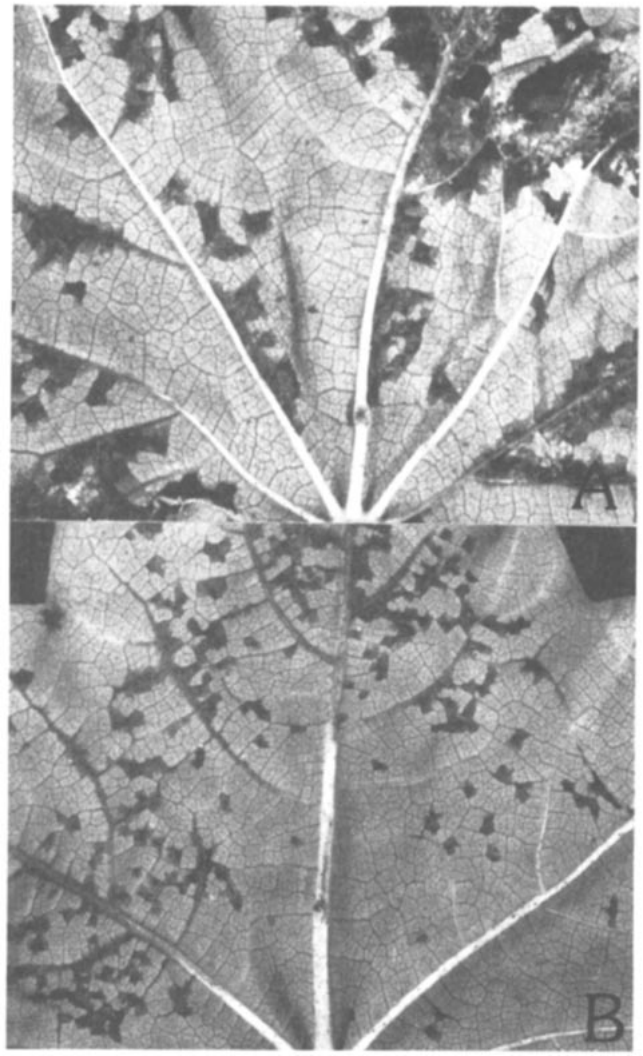

Fig. 1. A) Bacterial blight susceptible reaction of Acala 44 and B) Intermediate resistance reaction of the mutant, Westburn 70-M2.

Table 2. Comparative resistant (−) or susceptible (+) reactions on upland cotton bacterial blight differentials and the mutant Westburn 70-M2 with several races and isolates of *X. malvacearum*.

Bacterial blight culture	Upland line and resistance gene(s): Ac44 (none)	Ac121 (b_7)	Ac161 (B_N)	Ac13 (B_2)	AcB_3 (B_3)	AcB_4 (B_4)	AcB_5 (B_5)	Im216 (Several)†	Wb70-M2 (?)
Race									
1	+	−	−	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−	−
4	+	−	+	−	−	−	−	−	−
"ACCO 10"	+	+	+	+	+	+	+	−	+
Isolate									
From AcB_3	+	+	−	−	+	−	−	−	−
From AcB_4	+	+	−	−	−	+	+	−	−
From AcB_5	+	−	−	−	−	+	+	−	−

† See Brinkerhoff and Verhalen (5).

Table 3. Comparative lesion development from mutants of race 1 of *X. malvacearum* on upland cotton bacterial blight differentials and the mutant Westburn 70-M2.

Upland line	Resistance gene(s)	"Mutant-type" lesions†
Acala 121	b_7	+
Acala 161	B_N	+
Acala 13	B_2	−
Acala B_3	B_3	+
Acala B_4	B_4	+
Acala B_5	B_5	+
Im216	Several‡	−
(Acala 44 × Im216) F_8	Several‡	−
Westburn 70-M2	(?)	−

† Usually occur as a few late developing, water-soaked, susceptible-type lesions on leaves that initially appear resistant (3, 4).
‡ See Brinkerhoff and Verhalen (5).

Mexico and Oklahoma. However, those effects are likely *not* pleiotropic with the gene for blight resistance because the M_5 blight resistant selection was normal.

The relationship of this mutant to previously identified genes for bacterial blight resistance (4) has yet to be fully established. Inoculations with races 1, 2, 4, and "ACCO 10" and three isolates obtained from resistant host lines (but unidentified as to race) show that the mutant is not identical with the blight-resistance genes B_3, B_4, B_5, b_7, nor B_N (Table 2). Furthermore, the failure of "mutant-type" lesions to develop on Westburn 70-M2 from race 1 also indicated that the mutant was not identical to blight-resistance genes B_3, B_4, B_5, b_7, or B_N (Table 3). Neither line of evidence eliminates the possibility that it could be gene B_2; but results (indicating segregation or non-segregation) in that decisive F_2 population are needed to establish such a relationship, if any. The disease reaction of the mutant is somewhat similar to that of the single genes B_2 or B_6 in an Acala 44 genetic background (Brinkerhoff, unpublished data). Other possibilities not yet tested include B_1, B_6, B_8, B_{9K}, B_{10K}, B_{9L}, B_{10L}, B_{11}, B_{In}, and B_S (4). Not all these genes are available in *G. hirsutum*; and several of those present in the species are not available in the single-gene tester lines required for genetic studies.

Mutation breeding appears useful in generating new sources of bacterial blight resistance in cotton. Intermediate levels of resistance to this disease in this crop are usually conditioned by single genes with major effects, large plant populations are easily handled; and efficient inoculating and screening techniques are available which eliminate escapes. Immunity can be obtained by combining certain single genes together onto a polygenic background (1, 2, 4, 5).

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