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Influence of Male Sterile and Normal Cytoplasms on the Expression of Bacterial Blight in Cotton Hybrids[1]

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ABSTRACT

Seedlings of 28 F_1 hybrids of *Gossypium* obtained from all single-cross combinations of seven male and four female parents were evaluated in a greenhouse for bacterial blight (caused by *Xanthomonas malvacearum* (E. F. Sm.) Dowson) resistance. The four female parents were two lines of male-sterile (*Gossypium harknessii* Brandagee × *G. hirsutum* L.) cytoplasm and their respective 'B' lines with normal (*G. hirsutum*) cytoplasm. Superimposed on each cytoplasm was a line resistant to bacterial blight and a susceptible line. *G. Harknessii* cytoplasm slightly enhanced bacterial blight resistance in F_1 hybrids, accounting for approximately 12% of the total resistance. This is the first reported case in cotton where cytoplasms have been demonstrated to influence the severity of a disease. Both cytoplasms remained stable for blight reaction over four environments. Resistant *G. harknessii* and *G. hirsutum* female parents combined well with all male lines to produce relatively high levels of blight resistance. Specific combining ability was probably due to partial dominance effects. Large environmental changes were apparently required to cause noticeable changes in blight reaction.

***Additional index words:* *Xanthomonas malvacearum* (E. F. Sm.) Dowson, Blackarm, Angular leaf spot, Combining ability, Dominance, Host-plant-resistance.**

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CYTOPLASMIC male sterility in various crops has greatly facilitated economic development of hybrids. Of major concern, however, is the occasional occurrence of cytoplasmically related disease susceptibility. The classic example of an association between cytoplasm and disease reaction in crops was the occurrence of southern corn leaf blight (SCLB), incited by *Helminthosporium maydis* Nisikado and Miyake, on maize (*Zea mays* L.) carrying the Texas (T) cytoplasm (5, 19, 21). SCLB caused extensive damage throughout the southern and midwestern states in 1969. Similar associations of cytoplasms with disease susceptibility have occurred with numberous other crop plants (4, 6, 7, 8, 14, 16, 18, 22).

The National Academy of Sciences (15, page 1), in reference to the SCLB epidemic, posed the question "How uniform genetically are other crops upon which the nation depends, and how vulnerable, therefore, are they to epidemics?" Presently, there is no imminent threat of a cotton (*Gossypium hirsutum* L.) disease epidemic, but the possibility certainly exists. Production of cotton, like corn, involves vast acreages, and most cotton cultivars share a nearly identical cytoplasm (11).

Investigations have been conducted to develop cytoplasmic male sterility in cotton (9, 13). Heritable cytoplasmic male sterility has been developed from crosses of Upland cotton (*G. hirsutum*) onto cytoplasm

from *G. harknessii* Brandagee (12). Fertility-restorer stocks are also being developed from male-fertile segregants taken from early backcross generations of the same cross (10). By use of the mechanism present in (*G. harknessii* × *G. hirsutum*^{n}), commercial exploitation of heterosis may be achieved (17, 20).

The purpose of this study was to investigate the effects of the *G. harknessii* cytoplasm on the expression of bacterial blight (*Xanthomonax malvacearum* (E. F. Sm.) Dowson) resistance in F_1 hybrids in comparison with normal *G. hirsutum* cytoplasm. This involved the determination of specific combining ability for blight reaction of lines with *G. harknessii* cytoplasm and their response to environments. Determination of the combining ability of certain pollinator lines was also desired. The knowledge gained in this study should aid cotton breeders in making decisions concerning the utilization of hybrids developed from the *G. harknessii* cytoplasm.

MATERIALS AND METHODS

Parents consisted of four female and seven male lines. Two male-sterile female lines with *G. harknessii* cytoplasm were previously developed from strains obtained from Vesta G. Meyer, Delta Branch Experiment Station, Stoneville, Miss., in 1971. Strains 1-8 and 'Acala 4-42' were selected as representative resistant and susceptible lines, respectively, having normal (*G. hirsutum*) cytoplasm. Both 1-8 and Acala 4-42 were backcrossed two times to the male-sterile source line to produce, respectively, uniform bacterial blight resistant and susceptible lines with *G. harknessii* cytoplasm. Throughout this paper, phenotype refers to the blight reaction (resistant or susceptible) of these four female parents. The seven male lines consisted of 'Pima S-4' Pima 8, E1124, E1097, B_2B_6 × Pima 32, 'Albar 637', and K0210. The first four are susceptible, and the latter three are resistant. Pima S-4 is a commercial cultivar of *G. barbadense* L. and Albar 637 a commercial *G. hirsutum* from Africa. Pima 8 is an experimental *G. barbadense* obtained from C. V. Feaster of the Cotton Research Center, Phoenix, Ariz. K0210 is a wild accession of *G. barbadense* obtained from E. L. Turcotte also of the Cotton Research Center B_2B_6 × Pima 32, E1124, and E1097 are experimental *G. barbadense* strains obtained from C. F. Chew, USDA-ARS plant pathologist (retired), New Mexico State Univ. The two latter lines were developed by E. F. Young, USDA-ARS, El Paso, Tex.

All single-cross combinations were made by hand emasculation to obtain F_1 hybrids during the summer of 1975. The F_1 hybrids were grown in a randomized complete block experimental design in a greenhouse. Seed was planted in peat pots in metal greenhouse flats. In planting the test material, 10 peat pots were randomly selected within each block for each experimental F_1 line. Two seeds were planted in each pot and later thinned to one plant. Two complete blocks were grown and evaluated in January and again in March 1976.

The bacterial blight, *X. malvacearum*, inoculum consisted of an equal mixtures of Races 2, 10, and 'Tularosa'. The latter is a highly virulent culture isolated by C. F. Chew in 1963-64. These races were mixed to obtain an inoculum of moderate pathogenicity without risk of excessively mild or severe reactions (3). The inoculum suspension was standardized by use of a spectrophotometer set at a wavelength of 355 m and by diluting with distilled water to obtain a transmittance of 21%. The number of viable cells in this inoculum mixture was approximately 4×10^n/ml as determined by serial dilution culturing.

The plants were inoculated when the majority of the cotyledons were fully expanded (approximately 10 to 13 days after planting). Inoculation was accomplished by use of a Speed Ball #B-6 pen to transfer the inoculum to the plants (2). This pen was modified to provide two parallel scratch marks in the lower epidermal cotyledon tissue and to distribute a uniform flow of inoculum to the scratch area. The modification consisted of spreading the prongs to 1.5 mm and adjusting the flow mechanism.

Table 1. Analysis of variance to determine the influence of main effects and interactions upon blight reaction.

Source	d.f.	MS
Environments	3	6.24 **
Dates	1	17.87 **
Environments within dates	2	0.43 **
Female Parents	3	36.43 **
Cytotypes (*G. hirsutum* vs. *G. harknesii*)	1	1.59 **
Phenotypes (resistant vs. susceptible)	1	107.61 **
Cytotypes × phenotypes	1	0.09 NS
Male parents	6	6.41 **
Females × males	18	2.44 **
Environments × females	9	0.43 **
Environments × cytotypes	3	0.04 NS
Environments × phenotypes	3	1.17 **
Environments × (cytotypes × phenotypes)	3	0.10 NS
Environments × males	18	0.10 *

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

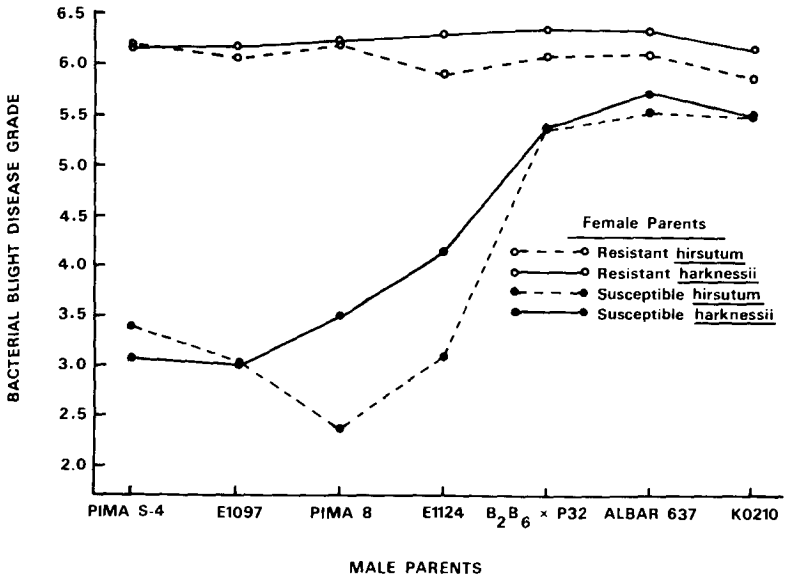

Fig. 1. Average bacterial blight reaction of F_1 hybrids produced from crossing four females × seven males.

To obtain favorable environmental conditions for bacterial blight expression, a cheesecloth cage approximately 30.5 cm high was constructed over the entire greenhouse bench. This cage provided a type of translucent humidity chamber for this experiment.

Observations of the blight reactions were made when the symptoms appeared to have reached their maximum expression (usually 9 to 13 days after inoculation). Accumulated heat units (1), as measured by a hygrothermograph monitoring each block, were used instead of time duration as a guide to inoculation and reading on each block. A nine-grade system was used to determine blight reaction based on observations of the size and appearance of lesions; grade 9 being completely resistant (2). The blight readings of all plants (or all that emerged and grew) were used for estimating the disease grade of each experimental F_1 line. Means were analyzed statistically by use of seven (males) $\times$ two (cytotypes) $\times$ two (phenotypes) $\times$ four (two dates $\times$ two environments) factorial set of treatments.

RESULTS AND DISCUSSION

The data (Table 1), show that nearly all variability was due to the phenotype effect of the female parents, as expected from the deliberate design of the experiment. However, the cytotype effect was also found to be highly significant. The mean value for *G. harknessii* (+5.29) was significantly higher than that for *G. hirsutum* (+5.05), indicating that the *G. harknessii* cytoplasm slightly enhanced the expression of bacterial blight resistance in the F_1 hybrids. This gave assurance that *G. harknessii* male-sterile cytoplasm did not condition bacterial blight susceptibility in F_1 hybrids.

The lack of significant cytotype $\times$ phenotype interaction suggests that the presence or absence of resistance genes, to bacterial blight races A, 10, and 'Tularosa', does not have an effect on *G. harknessii* cytoplasm or vice versa.

Female parents significantly interacted with male parents (Table 1). More descriptive results may be obtained from Fig. 1. The resistant *G. harknessii* and *G. hirsutum* female parents showed high general combining ability for blight reaction. This was indicated by both resistant female parents combining uniformly well with all male parents to provide relatively high levels of blight resistance. Susceptible *G. harknessii* and *G. hirsutum* female parents showed high specific combining ability for blight reaction. This was especially evident with the resistant pollinators. Disease grades for the original female parents were 6.03, 6.23, 2.78, and 4.33 for the resistant *G. hirsutum* and *G. harknessii* and the susceptible *G. hirsutum* and *G. harknessii,* respectively. Therefore, specific combining ability was due to dominance effects. Partial, rather than complete dominance, was reflected (Fig. 1). These results also indicate that one resistant parent, either male or female, is sufficient to assure blight resistance in F_1 hybrids.

A nonsignificant environment $\times$ cytotype interaction showed that the cytoplasms of both *G. harknessii* and *G. hirsutum* are stable over the environments encountered in this test (Table 1). Resistant male parents B_2B_6 $\times$ Pima 32, Albar 637, and K0210 showed no difference in combining abilities, indicating equal effectiveness as sources of resistance.

CONCLUSIONS

The knowledge gained in this study should aid breeders in forecasting the stability of bacterial blight resistance in future cotton hybrids developed from the *G. harknessii* cytoplasmic male sterile system. Results presented herein indicate that *G. harknessii* cytoplasm has no susceptibility reaction to bacterial blight, is stable over environmental conditions, and combines well for relatively high levels of resistance.

There is a valid cause for concern that there should be cytoplasmic as well as genetic diversity with respect to host plant resistance (11, 15). Apparently this is the first documentation of a significant cytoplasmic influence on resistance to a major disease in cotton. Information on cytoplasmic reaction to many diseases is very important to breeders of hybrid cotton. Additional studies are needed to study the cytoplasmic response to other diseases, including nuclear-cytoplasmic interactions. In the case of *X. malvacearum,* genes for resistance were effective in both cytoplasms tested, but the possibility that specific genes for resistance to other diseases may not be equally effective in certain cytoplasms should not be overlooked. Strong cytoplasmic resistance to any disease could be an asset in conventional breeding also (11).

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