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Quantitative analysis of resistance in cotton to three new isolates of the bacterial blight pathogen *

T.P. Wallace ** **and K.M. El-Zik**

Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843, USA

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Summary. Genetic variability for virulence of the bacterial blight pathogen [*Xanthomonas campestris* pv *malvacearum* (Smith) Dye] on cotton (*Gossypium hirsutum* L.) has been shown by the identification of 19 races of the pathogen based on disease reactions of a set of ten host differentials. This study was conducted to determine the inheritance of host resistance to three recently identified isolates of *X. campestris* pv *malvacearum*, which are virulent on the entire set of differentials. True leaves of Tamcot CAMD-E, LEBOCAS-3-80, Stoneville 825, and their F_1, F_2, and backcross progenies were wound-inoculated in the field with separate bacterial suspensions of the virulent HV3, HV7, and Sudan isolates of the pathogen. LEBOCAS-3-80 was replaced with S295, a new immune cultivar, for a greenhouse study in which both cotyledons and true leaves were inoculated. Disease reactions were rated on a scale of 1–10, and genetic models were proposed utilizing generation means analysis. Dominance, when significant, was in the direction of resistance in all but one cross-isolate combination. Digenic interaction components indicated a duplicate type. Narrow-sense heritability for resistance ranged from 0.59 to 0.68; therefore, primarily additive-genetic variability among the selected cutlivars was detected, indicating that breeding for improved resistance to these isolates is a practical goal.

Key words: *Xanthomonas campestris* pv *malvacearum* – *Gossypium hirsutum* L. – Resistance – Inheritance – Gene Action

Introduction

Bacterial blight of cotton can affect all stages and plant parts of the cotton plant throughout the growing season. The disease can be particularly destructive to the long-staple, high quality tetraploid cottons of *Gossypium barbadense* L., and also attacks the tetraploid Upland cottons, *G. hirsutum* L. (Brinkerhoff 1963).

The genetic variability of *Xanthomonas campestris* pv *malvacearum* (Smith) Dye for virulence and other traits has been well documented (Arnold and Brown 1968; Brinkerhoff 1963; Brinkerhoff et al. 1984; Cross 1963). The Cotton Disease Council (USA) currently recognizes 19 races of *X. campestris* pv *malvacearum* based on disease reactions of a set of ten host differentials (Bird 1986). Cultivars immune to all the USA races of *X. campestris* pv *malvacearum* have been developed using a mixture of four races of the pathogen to identify sources of resistance (Bird 1982; Bird et al. 1984). The majority of genes identified have been described as partially to completely dominant for resistance (Bird and Hadley 1958; El-Zik and Bird 1970; Green and Brinkerhoff 1956; Knight 1957, 1963). Immunity to all the USA races of *X. campestris* pv *malvacearum* conferred by the $B_2B_3B_{sm}$ gene combination has been stable for over 20 years (Bird 1986).

Follin (1981) reported the appearance of several strains of *X. campestris* pv. *malvacearum* in Burkina Faso that were virulent on the entire set of host differentials. A survey of races present in western and central Africa subsequently revealed that a population exists in both Burkina Faso and Chad, which can overcome all the major genes for bacterial blight resistance currently used in USA breeding programs (Follin 1983). These new isolates were designated HV1, HV3, and HV7. Another isolate, discovered in Sudan in 1983, was reported as

* Contribution of the Department of Soil and Crop Sciences and the Texas Agricultural Experiment Station

** Current address: Department of Agronomy, P.O. Box 5248, Mississippi State, MS 39762, USA

being virulent on the B_2B_3 gene combination (Bird 1986). In 1986, a new cultivar developed at the Bebedjia station in Chad, designated S295, was reported to be resistant to the isolates capable of overcoming resistance conferred by the B_2B_3 or $B_{9L}B_{10L}$ gene combinations (Girardot et al. 1986). Resistance to the HV1 isolate in S295 was found to be conferred by a single gene with complete dominance for resistance (Wallace and El-Zik 1989). Varying levels of resistance to the HV3, HV7, and Sudan isolates have been reported (Follin 1983). The objective of this study was to determine gene action and to investigate the inheritance of resistance to the HV3, HV7, and Sudan isolates in selected cotton cultivars and strains. This information will be useful to breeders in developing bacterial blight-resistant germ plasm.

Materials and methods

Three parents were selected and a half-diallel mating scheme was initiated to produce F_1, F_2, and backcross progenies. The following year seed of the S295 variety, which is resistant to the Sudan and HV African isolates, was obtained, and a second set of parents and their progenies was developed. The first parental set included 'Tamcot CAMD-E' (CAMDE), 'LEBOCAS-3-80' (LEBO), and 'Stoneville 825' (ST825). CAMDE and LEBO were selected because of their high level of resistance to the USA races of *X. campestris* pv *malvacearum*. 'ST825' is susceptible to the USA races of the pathogen. 'S295' was chosen for its high level of resistance to all known races and isolates of *X. campestris* pv *malvacearum*. In the second parental set, LEBO was replaced with S295 to obtain a set of parents representing the full range of disease reactions from highly resistant to fully susceptible. Hybridization and selfing of the 3 parents produced 3 F_1s, 3 F_2s, and 6 backcrosses for a total of 15 populations for each set of parents.

Each isolate of *X. campestris* pv *malvacearum* was cultured on potato carrot dextrose agar media (PCDA) and maintained at 22 °C. Inoculum was prepared from 5 to 7-day-old cultures by placing a small portion of the bacterial growth inside a small glass vial and diluting it with sterile water. The bacterial suspension was adjusted to produce an inoculum density of approximately 1.0×10^6 bacteria/ml. True leaves were inoculated in the field experiment, while both cotyledons and true leaves were inoculated in a greenhouse experiment. Fully expanded cotyledons of seedlings and the top two main stem leaves at nodes 7–9 were inoculated using the toothpick scratch method (Bird 1982). Plants were evaluated for disease reaction 15–21 days after inoculation, depending upon disease development. Disease expression was graded on the scale of 1 (high resistance) to 10 (fully susceptible) described by Bird and Hadley (1958).

The first parental set was evaluated in the field and the second parental set was evaluated in the greenhouse. Entries for both field and greenhouse experiments were arranged in randomized complete block designs. The field experiment consisted of 15 populations with 4 replications. Plots were 12.2 m in length, 1 m wide, with 10-cm plant spacing. Parental, F_1, and backcross generations were grown in one-row plots and F_2 progenies in two-row plots. For parental and F_1 entries, 10 plants/plot were inoculated, and 25 plants/plot were inoculated for both F_2 and backcross entries. For the greenhouse experiment, entries from the second parental set were grown in 30-cm pots. A single greenhouse bench represented one of seven replications. Three plants per replication were inoculated for parental and F_1 populations, 27 for F_2, and 12 plants for backcross populations.

An analysis of variance using plot means was performed on disease grades for parental and backcross generations within each cross, to aid in deciding which cross and isolate combinations justified a genetic analysis. Frequency histograms were constructed for each entry and isolate, and were examined for any shifts in the median disease grade to determine their suitability for genetic analysis. In crosses where parents and backcrosses differed and F_2 distributions indicated quantitative inheritance, generation means analysis as described by Mather and Jinks (1971, 1977) was employed.

The method described by Rowe and Alexander (1980) for computer application of the joint scaling test was utilized for estimating genetic parameters by way of matrix inversion. A simple additive-dominance model was tested first and, if found to be inadequate, the model was extended to include digenic interaction components.

Narrow-sense heritability estimates were calculated using the method described by Warner (1952) for cross-isolate combinations, which indicated that an additive-dominance model, excluding non-allelic interactions, was adequate. The potence ratio, a measure of average degree of dominance described by Mather and Jinks (1977), was also calculated for simple additive-dominance models. Broad-sense heritability estimates were calculated for the remaining cross-isolate combinations showing quantitative inheritance, using the formula given by Briggs and Knowles (1967).

Results and discussion

Field experiment

True leaf disease grades ranged from 2.7 to 5.9 for parental cultivars and from 2.8 to 5.6 for F_1 progenies grown in the field (Table 1). The absence of higher disease grades (7–10) suggests that the isolates were not highly aggressive under field conditions on these particular genotypes. The analysis of variance and frequency histograms of parental and backcross entries within each cross indicated significant differences between CAMDE and LEBO for disease reaction to each isolate. Parental cultivar LEBO expressed a greater level of resistance

Table 1. True leaf disease grades for parental and F_1 populations inoculated in the field with HV3, HV7, and Sudan isolates of *Xanthomonas campestris* pv *malvacearum*

Isolate	Parental means			F_1 means		
	CAMDE	LEBO	ST825	CAMDE × LEBO	CAMDE × ST825	LEBO × ST825
	Disease grade[a]					
HV3	4.1	2.9	4.2	2.9	4.3	3.1
HV7	5.9	4.6	5.4	5.1	5.6	4.6
Sudan	3.9	2.7	3.8	3.2	3.5	2.8

[a] Grades based on a scale of 1 (highly resistant) to 10 (fully susceptible)

than CAMDE for each isolate. When generation means analysis was applied to HV3 true leaf disease grades for this cross, an adequate genetic model was not obtained (Table 2). Parental and F_1 means for this cross, however, indicated a degree of dominance in LEBO for resistance to the HV3 isolate. Simple additivity of the individual genes contributing to the additive (*d*) and dominance (*h*) components, an assumption of the model, may have been violated, or the differences among generation means were too small to evaluate.

A simple additive-dominance model proved inadequate for true leaf disease grades for the CAMDE × LEBO cross when inoculated with the HV7 isolate. When extended to include digenic interactions, a five-component model yielded a nonsignificant Chi-square (Table 2). The additive × dominance component (*j*) was the only nonsignificant parameter. A relatively large *h* component indicated that dominance was important. The negative sign of *h* indicated dominance was in the direction of resistance. The signs of *h* and *l* (*h* × *h* digenic interaction) suggest interaction mainly of the duplicate type (Mather 1967). A significant interaction component violates the assumptions for the estimation of additive variance in the calculation of narrow-sense heritability; therefore, it was not estimated. Broad-sense heritability, however, was found to be 0.60.

Table 2. Summary of generation means analysis for each cross-isolate combination for the field and greenhouse experiments

Cross	Isolate		
	HV3	HV7	Sudan
	Genetic parameter[a]		
CAMDE × LEBO			
Field	–	*m d* −*h* −*i l*	*m d* −*h l*
LEBO × ST825			
Field	*m d* −*h*	–	–
CAMDE × ST825			
Field	–	–	–
Cotyledons	–	–	–
True leaves	–	–	–
CAMDE × S295			
Cotyledons	*m d* −*h*	*m d h*	–
True leaves	*m d* −*h* −*i* −*j l*	*m d* −*h* −*i* −*j l*	–
ST825 × S295			
Cotyledons	*m d* −*h* −*i l*	*m d* −*h* −*i* −*j l*	–
True leaves	*m d* −*h*	–	–

[a] Genetic parameters are *m* – mean, *d* – additive, *h* – dominance, *i* – *d* × *d* interaction, *j* – *d* × *h* interaction, and *l* – *h* × *h* interaction components

Generation means analysis of true leaf disease grades for CAMDE × LEBO, inoculated with the Sudan isolate, indicated that a four-component model with *m*, *d*, *h*, and a significant dominance × dominance (*l*) component could explain the differences observed in generation means (Table 2). The signs of *h* and *l* indicated dominance in the direction of resistance and a duplicate type of interaction. The estimate for broad-sense heritability was negative. Interaction of the duplicate type can reduce the variance of the F_2 generation below that which would occur in the absence of interaction; this would tend to reduce broad-sense heritability (Mather and Jinks 1971).

Analysis of variance for the LEBO × ST825 cross indicated significant differences between parents and backcrosses, when inoculated with the HV3 isolate but not with the HV7 and Sudan isolates. Parental and F_1 means for the LEBO × ST825 cross inoculated with the HV3 isolate suggested resistance of a quantitative nature (Table 1). The three-component additive-dominance model was found to be adequate, indicating the absence of interactions (Table 2). Dominance was in the direction of resistance and appeared to be less significant than the additive component. The potence ratio was found to be 0.63 and narrow-sense heritability 0.65.

Parental cultivars CAMDE and ST825 reacted with near equal disease grades when inoculated with the HV3, HV7, or Sudan isolates (Table 1). No significant differences between the two parents were observed for any of the isolates, and a genetic analysis was not attempted.

In general, CAMDE and ST825 responded similarly to the three isolates and were less resistant than LEBO. Of the three isolates, inoculation with HV7 resulted in higher disease grades for all three cultivars. When an adequate genetic model was obtained, dominance was in the direction of resistance.

Greenhouse experiment

Both cotyledons and true leaves of the second parental set were evaluated in the greenhouse. Cotyledon and true leaf disease grades ranged from 2.1 to 4.1 and from 1.7 to 3.9, respectively, for parental cultivars (Table 3). Analysis of parental and backcross means for the cross common to both field and greenhouse studies, CAMDE × ST825, indicated that there were no differences among the parents for resistance to any of the three isolates. Cotyledon and true leaf disease grades were similar for both CAMDE and ST825. True leaf disease grades, however, were slightly lower than cotyledon disease grades for both the HV3 and HV7 isolate (Table 3). The mature true leaf was less susceptible than cotyledons to the pathogen. Similar results were observed for this cross under field conditions.

When cotyledons were incoulated with the HV3 isolate, CAMDE displayed evidence of heterogeneity.

Table 3. Cotyledon and true leaf disease grades for parental and F_1 populations inoculated in the greenhouse with HV3, HV7, and Sudan isolates of *Xanthomonas campestris* pv *malvacearum*

Isolate	Parental means			F_1 means		
	CAMDE	ST825	S295	CAMDE × ST825	CAMDE × S295	ST825 × S295
	Disease grade[a]					
HV3						
Cotyledon	3.5	3.0	2.1	3.4	2.2	2.0
True leaf	3.0	2.9	2.2	3.1	2.7	2.3
HV7						
Cotyledon	4.1	3.9	2.5	4.0	2.8	2.2
True leaf	2.4	2.4	1.7	2.3	2.4	1.8
Sudan						
Cotyledon	3.3	3.2	3.5	3.1	3.5	3.3
True leaf	3.8	3.9	3.7	3.8	4.2	3.9

[a] Grades based on a scale of 1 (highly resistant) to 10 (fully susceptible)

Cotyledon disease grades ranged from 1 to 6, with 77% of the plants falling into grades 3 and 4. Cotyledon disease grades for S295, however, ranged from 1 to 3, with 86% of the plants falling into grade 2. Despite the apparent heterogeneity of CAMDE for reaction to the HV3 isolate, means and frequency distributions of F_2 and backcross generations from the CAMDE × S295 cross indicated dominance in S295 for resistance to the HV3 isolate. A simple additive-dominance model produced a nonsignificant Chi-square, indicating the absence of nonallelic interactions (Table 2). Dominance was in the direction of resistance and was of the same relative magnitude as the additive component. Estimation of the additive portion of variance produced a narrow-sense heritability estimate of 0.68; the potence ratio was 0.72. Plants of CAMDE appeared to be more homogeneous when true leaves were inoculated with the HV3 isolate. The differences in the two backcrosses for reaction of true leaves were very small compared to those for cotyledons. A simple additive-dominance model for true leaf disease grades was found inadequate for explaining differences among generation means. A perfect fit estimate of the model with digenic interactions indicated that each component was significantly different from zero (Table 2). With zero degrees of freedom remaining, the model could not be tested for goodness of fit. It appears, however, that interaction of the duplicate type is present, and dominance is in the direction of resistance. Heritability in the broad sense for resistance of true leaves to the HV3 isolate was 0.52.

The parental cultivar CAMDE also appeared heterogenous when cotyledons were graded for resistance to the HV7 isolate. Two plants had grades of 9 and 10, with the remaining plants falling into grades 3–6. S295 had all plants falling into grades 2 and 3. Despite the apparent heterogeneity of CAMDE, the F_1 and backcross frequency distributions indicated dominance for resistance to the HV7 isolate in S295. Parental and F_1 cotyledon disease grades also indicated dominance for resistance in S295 to HV7 (Table 3). A simple additive-dominance model was adequate for explaining the differences among generation means (Table 2). However, a narrow-sense heritability estimate of 1.7 indicated that the heterogeneity of CAMDE may have influenced the variance of the F_2 generation, resulting in an inflated estimate of the additive proportion of variance. The assumption of equal environmental × generation variances among the different generations also may have been violated and may have influenced the estimate of heritability. The dominance component (h) was positive, indicating dominance in the direction of susceptibility, and further implicating the effects of a heterogeneous parental cultivar.

When true leaves were inoculated with HV7, CAMDE was much more homogenous, with 85% of the plants falling into grades 2 and 3. However, S295 was more resistant, with 95% of the plants falling into grades 1 and 2. Slight differences among the two backcrosses indicated that S295 was more resistant to the HV7 isolate than CAMDE. A six-component perfect-fit estimate indicated the presence of digenic interactions, with all three interaction components being significant (Table 2). The model could not be tested for goodness of fit, so the possibility of higher order interactions could not be excluded. The dominance component was in the direction of resistance, and the interaction appeared to be of the duplicate type as indicated by the signs of h and l. Broad-

sense heritability was 0.24. No differences were detected among the two parental cultivars for this cross when inoculated with the Sudan isolate.

Inoculation of cotyledons with the HV3 isolate indicated that S295 was slightly more resistant than ST825, and F_1 means indicated a degree of dominance for resistance in S295 (Table 3). A simple additive-dominance model was not adequate for explaining the differences among generation means, but a perfect-fit six-component estimate yielded a five-component model with a nonsignificant Chi-square value (Table 2). The dominance (*h*) and additive (*d*) components were significant, with dominance in the direction of resistance. The additive × additive (*i*) and dominance × dominance (*l*) interactions were of the same magnitude and in opposite directions. The signs of *h* and *l* are opposing and indicate mainly a duplicate type of interaction. Only broad-sense heritability was applicable, and was estimated to be 0.78. S295 expressed a slightly higher level of resistance (grade 2.2) than ST825 (2.9) to the HV3 isolate in the true leaf stage (Table 3). For true leaf disease grades, a simple additive-dominance model could explain the differences among generation means (Table 2). Dominance was in the direction of resistance and was of the same magnitude as the additive component. The potence ratio was 0.73 and narrow-sense heritability was 0.59.

Cotyledons of S295 also were more resistant than ST825 to the HV7 isolate. A perfect-fit estimate of the six parameters failed to reveal any nonsignificant interaction components (Table 2). With zero degrees of freedom remaining, a Chi-square test for goodness of fit was not possible. The most important epistatic component was the dominance × dominance interaction, and deviation was in the direction of susceptibility. Dominance was in the direction of resistance. Broad-sense heritability was 0.42. True leaves of S295 also were more resistant (grade 1.7) than those of ST825 (grade 2.4) when inoculated with the HV7 isolate. Generation means analysis of true leaves also required fitting the six-component model, but the additive × dominance component (*j*) was nonsignificant, thus allowing a Chi-square test for goodness of fit to a five-component model. The remaining components *m*, *d*, *h*, *i*, and *l* were significant, but the Chi-square test for the model also was significant, suggesting the possibility of higher order interactions. Inoculation with the Sudan isolate failed to distinguish any differences between ST825 and S295 in both cotyledons and true leaves. Both parents and progenies were resistant to the Sudan isolate.

Conclusions

Significant additive variation indicates that breeding for improvement in resistance to isolates of *X. campestris* pv *malvacearum* should be possible. The low average disease grades resulting from inoculating with these isolates under field conditions, however, suggest that the isolates have a low level of aggressiveness, or that the genotypes examined possess a level of horizontal resistance. Disease resistance of true leaves was higher compared to cotyledons, and could be the result of a physiological change in the host or a change in the genetic system controlling resistance. Inoculation at both the cotyledon and mature leaf stage would provide a more reliable evaluation of a particular genotype for resistance to *X. campestris* pv *malvacearum*.

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