EVALUATION OF COTTON CULTIVARS AND BREEDING LINES FOR RESISTANCE TO BACTERIAL BLIGHT

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Abstract

Bacterial blight (*Xanthomonas axonopodis* pv. *malvacearum*) is one of the most serious diseases in cotton production in the US and worldwide. In this greenhouse study, a total of 128 commercial cotton cultivars and elite breeding lines and 339 obsolete cultivars and lines were evaluated in replicated tests. FM 9058F, FM 2334GLT, FM 1830GLT, FM 2484B2F, and PHY 375WRF were used as resistance checks. One cotyledon from each seedling was inoculated with Xam inoculum using a toothpick method, while another cotyledon was scratched with water as a negative control. In addition to the above five commercial transgenic cultivars, three elite lines (Ark 0409-3, Ark 0409-7, and LA111038), two cultivars (FM 958, FM 2322GLT, and PHY 339 WRF), and 49 obsolete cultivars and lines were resistant.

Introduction

Bacterial blight (*Xanthomonas axonopodis* pv. *malvacearum*, Xam) is a serious disease in cotton. Currently, 19 bacterial blight races have been recognized based on 11 host differential lines (Hunter et al., 1968; Delannoy et al., 2005). Among these races, race 18 is the most virulent in the U.S. This disease is documented in almost every cotton-growing country in the world. The pathogen infects the aerial part of plants at all stages, and host responses may be either susceptible or resistant at different levels (Jalloul et al., 2015). Tissues in susceptible plants develop expanding water-soaked lesions that last one week or longer after infection. However, responses in resistant tissues show a hypersensitive reaction that leads to death of a limited number of leaf cells in the inoculated or infected area within two days (Delannoy et al., 2005; Jalloul et al., 2015). A naturally resistant genotype to Xam in cotton was first confirmed in 1939, when work on breeding cotton for Xam resistance began in tetraploid cotton species, through transfer of resistance (R) genes (i.e., genes that confer resistance to bacterial blight) from resistant species or genotypes of *Gossypium* (Delannoy et al., 2005). This strategy has been widely used due to the efficient role of blight-resistant cultivars in disease control (Bird et al., 1981). The tetraploid *G. hirsutum* (Upland) cultivars show the highest range of disease expression, varying from completely susceptible to highly resistant, while little resistance occurs naturally in another tetraploid *G. barbadense* (Delannoy et al., 2005). A total of 19 R genes for Xam resistance have been identified (Delannoy et al., 2005). The development of cultivars carrying one or more R genes that show little or no disease symptoms in the presence of the pathogen is a major goal of cotton breeders in many regions. However, little is known with regard to the resistance levels of modern U.S. cotton cultivars and current commercial cultivars. Thus, the objective of this study was to evaluate cotton cultivars and breeding lines for resistance to bacterial blight.

Material and Methods

This study was conducted in the greenhouse of the Cotton Breeding and Genetics Program at New Mexico State University, Las Cruces, NM in 2016. The genotypes tested were divided into different tests, designated 14RB (Regional Breeder's Testing Network), 14NV (Official Cotton Variety Test), 15H (Preliminary Yield Test-1), 15M (Preliminary Yield Test-2), and 15G (Association Mapping Panel). Each test was arranged in a randomized complete block design with 32 genotypes and 3 replications except for 339 genotypes and 2 replications in test 15G. Tests 14NV, 15H and 15 M were repeated once. In a subsequent study, 10 resistant cultivars from 14NV, 7

susceptible lines from 15H, 10 susceptible lines from 15M, and 68 genotypes (including 49 resistant and 17 susceptible ones) from 15G were selected for a retesting. The test was arranged in a randomized complete bock design with 3 replications. The following five resistant genotypes, i.e., FM 9058F, FM 2334GLT, FM 1830GLT, FM 2484B2F, and PHY 375WRF were used as resistance checks. Ten seed for each genotype was planted in a 4-inch pot as a replication. The Xam culture used was identified as race 18. The bacteria were cultured on the ATCC medium (carrot potato dextrose agar) at 30 °C for 24-48 h until they homogeneously covered the Petri dishes. Three weeks after planting, one cotyledon from each seedling was inoculated with the bacterial culture by the toothpick method (Bird, 1982), while another cotyledon was scratched with tap water as the negative control at the same time. The inoculated plants were maintained in 99 % relative humidity at 23-25 °C in a plastic box for 24-48 h in a lab before transferred back to the greenhouse. The seedlings from 14RB, 14NV, 15H, and 15M tests were evaluated for resistance 14 days post inoculation (dpi). For test 15G, plants were evaluated for bacterial blight resistance three times, i.e., 14 and 19 dpi on cotyledons and 42 dpi on true leaves. This time frame was adopted because mild symptoms continued to develop over time, and the infections were increased in the second and third evaluations. Plants with water-soaking symptoms were recognized and rated as bacterial blight susceptible, and plants that did not show water-soaking symptoms were considered resistant. As such, the disease sensitivity was rated as follows: 0 = resistant (showing a hypersensitive reaction) and 1= susceptible. The percentage of susceptible plants was used to calculate an average severity on a plot basis, i.e., - 0 for no susceptible plant, and 1 for 100% susceptible plants.

An analysis of variance (ANOVA) using SAS software version 9.4 was performed for each test (2012 SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The susceptible cotyledons showed water-soaking symptoms first, which led to chlorotic halos around the inoculation site. In contrast, the resistant cotyledons appeared as dried marks from the toothpick inoculation scratches (Fig. 1). Moreover, true leaves in susceptible plants showed angular leaf spots and blight vein symptoms, while true leaves in resistant genotypes showed no symptoms (Fig. 2). Seedlings with water-soaking cotyledons developed true leaves with angular leaf spots.

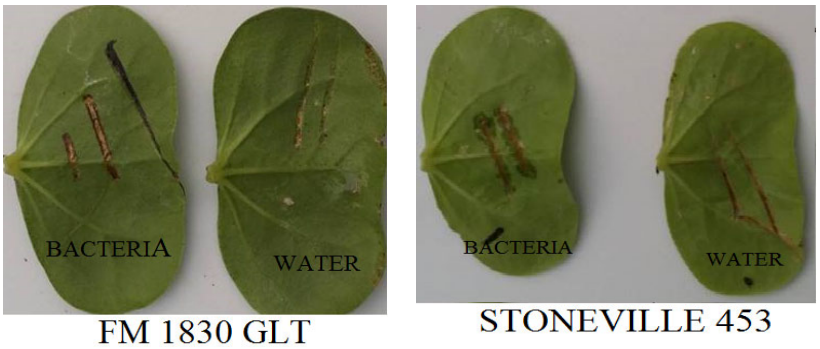

Fig. 1. Different responses in cotyledons between a resistant (left) and a susceptible (right) genotype.

Fig. 2. Different responses in TRUE leaves between a resistant (left) and a susceptible (right) genotype.

The analysis of variance (ANOVA) for each test with elite breeding lines and current commercial cultivars is shown in Table 1.

Table 1. An analysis of variance for bacterial blight resistance in each replicated test. 14NV, 14H and 14M were tested twice (e.g., 14NV-1 for the first test and 14NV-2 for the second test).

Test	Source	df	SS	MS	F value	P value
14RB	Block	3	0.78	0.26		
	Genotype	31	1.38	0.04	1.50	0.07
	Error	91	2.70	0.03		
14NV-1	Block	2	0.08	0.04		
	Genotype	31	1.33	0.04	4.20	< 0.0001
	Error	62	0.63	0.01		
15H-1	Block	2	0.06	0.03		
	Genotype	31	1.07	0.03	1.42	0.11
	Error	62	1.50	0.02		
15M-1	Block	2	0.43	0.21		
	Genotype	31	0.66	0.02	0.84	0.7
	Error	62	1.59	0.02		
14NV-2	Block	2	0.43	0.21		
	Genotype	31	8.82	0.28	4.56	0.0001
	Error	59	3.68	0.06		
15H-2	Block	2	0.68	0.34		
	Genotype	31	2.16	0.07	1.18	0.28
	Error	62	3.65	0.05		
15M-2	Block	2	1.30	0.65		
	Genotype	31	2.88	0.09	1.70	0.04
	Error	62	3.39	0.05		

With elite breeding lines from public breeding programs (14RB) in the U.S. and New Mexico State University (15H and 15M), no significant genotypic variations were detected (Table 2). However, 9 cultivars in the Official Variety Test (i.e., 14NV) showed resistance to bacterial blight (Table 2).

Table 2. Bacterial blight resistant lines and some selected susceptible lines in 14RB, 15H and 15M (Test 1 and Test 2), Las Cruces, NM, May 2016. 0- no susceptible plants, and 1- 100% susceptible plants.

Genotypes	Test 1	Test 2
FM 1830GLT	0.00	0.00
FM 2334GLT	0.00	0.00
FM 2484B2F	0.00	0.00
PHY 339WRF	0.00	0.00
Ark 0409-3	0.00	0.00
Ark 0409-7	0.00	0.00
FB BLEND	0.00	0.00
LA111038 (okra)	0.07	0.00
FM 958	0.00	0.00
FM 2322GL	0.68	1.00
13P1088	0.55	1.00
14S1177	0.06	0.26
14S1200	0.16	0.91
14S1212	0.17	0.44
14S1213	0.33	0.47
14S1214	0.35	0.64
14S1239	0.30	0.70
14S1262	0.21	0.54
14T1085	0.27	0.41
14T1088	0.27	0.62
14T1197	0.10	0.37
14T1223	0.47	0.91
14T1426	0.28	0.63
14T1433	0.26	0.57
Resistant controls		
FM 2334GLT	0.00	0.00
FM 9058F	0.00	0.00
FM 2484B2F	0.00	0.00
PHY 375WRF	0.00	0.00
FM 1830GLT	0.00	0.00

In the test with a diversity panel of 339 obsolete U.S. cotton cultivars and breeding lines (i.e., 15G), the ANOVA indicated a significant genotypic variation (Table 3). A total of 49 lines expressed high levels of resistance to bacterial blight (Table 4), while the rest of the genotypes displayed different levels of susceptibility.

Table 3. Analysis of variance of bacterial blight resistance in a diversity panel of 339 genotypes screened 13, 19, and 43 days post inoculation (dpi), Las Cruces, NM, August 2016.

Screening date	Source	df	SS	MS	F value	P value
13 dpi	Block	1	0.0004	0.0004		
	Genotype	338	73.5	0.217	10.34	< .0001
	Error	330	6.94	0.02		
19 dpi	Block	1	0.008	0.0084		
	Genotype	338	80.22	0.23	21.4	< .0001
	Error	330	3.66	0.011		
43 dpi	Block	1	0.44	0.44		
	Genotype	334	73.73	0.22	11.5	< .0001
	Error	288	5.5	0.019		

The resistant cultivars and lines and several susceptible ones were selected for a retest in the greenhouse, and almost the same results were obtained (Table 2 and 4).

Table 4. Evaluation of bacteria blight resistance in resistant lines and some susceptible lines in two tests (Test 1 and Test 2) of Trial 15G, Las Cruces, NM, August 2016. 0- no susceptible plants; and 1- 100% susceptible plants.

Genotype	Test 1	Test 2
Resistant genotypes		
ACALA 1064 (New Mexico)	0.00	0.00
ACALA 8	0.00	0.00
ARKOT 8102	0.00	0.33
ARKOT 8606	0.00	0.00
C5HUG2BES-2-87	0.00	0.00
CABCSV506S-1-94	0.00	0.00
CABD3CABCH-1-89	0.00	0.00
CABD3SHP3S-1-90	0.00	0.00
CAHUGLBBCS-1-88	0.00	0.00
CASCOT L-7	0.00	0.00
CD3HCABCUH-1-89	0.00	0.00
CD3HCAHUGH-2-88	0.00	0.00
CD3HHARCIH-1-88	0.00	0.00
CLEVEWILT 6 NAKED SEED	0.00	0.00
COKER 201	0.00	0.55
COKER'S WILDS #4	0.00	0.00
FM 966	0.00	0.00
GP 1005	0.00	0.00
GP 3755	0.00	0.00
GP 5479	0.00	0.00
GREEN LINT	0.00	0.00
GSC 27	0.00	0.00
H1330	0.00	0.00
HGPICG14QH-1-94	0.00	0.00
LAMBRIGHT 2020A	0.00	0.00
LANKART 511	0.00	0.00
LANKART 57	0.00	0.00
LBBCABCHUS-1-87	0.00	0.00
LBBCDBOAKH-1-90	0.00	0.00
M4	0.00	0.00
PAYMASTER HS200	0.00	0.00
PD 1	0.00	0.00
PD24HQBPIH-1-94	0.00	0.00
Pyramid	0.00	0.00
SA 2327	0.00	0.00
SA 2424	0.00	0.00
SG 747	0.00	0.00
SPNXCHGLBH-1-94	0.00	0.00
SPNXHQBPIS-1-94	0.00	0.00
TAM 86E-8	0.00	0.63

TAMCOT CAB-CS	0.00	0.00
TAMCOT CAMD-E	0.00	0.00
TAMCOT CD3H	0.00	0.00
TAMCOT PYRAMID	0.00	0.00
TAMCOT SP-21	0.00	0.00
TAMCOT SP-23	0.00	0.00
TAMCOT SP-37	0.00	0.00
TAMCOT SP-37H	0.00	0.00
TAMCOT SPHINX	0.00	0.00
Susceptible genotypes	0.81	1.00
ACALA 51		
ACALA NAKED SEED	0.76	1.00
ARK-2	0.42	0.73
BJAGL NECT	0.35	0.30
BLCABPD86S-1-90	0.50	0.00
DELTAPINE PREMA	1.00	1.00
DELTATYPE WEBBER	1.00	1.00
DES 119	1.00	1.00
DIXIE TRIUMPH	1.00	1.00
FTA	1.00	0.96
GSC 30	0.50	0.03
MAR5PD208S-4-90	0.50	0.30
MO-DEL	1.00	1.00
NC 88-95	0.53	0.64
STONEVILLE 453	0.78	0.69
TAM 87N-5	0.20	0.74
TIDEWATER (SEABROOKS) (G.B. INTO G.H.)	1.00	1.00
Resistant controls		
FM 2334 GLT	0.00	0.00
FM 9058F	0.00	0.00
FM 2484 B2F	0.00	0.00
PHY 375 WRF	0.00	0.00
FM 1830 GLT	0.00	0.00

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