Host Plant Resistance to Pathogens in MAR Cotton Germplasm

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ABSTRACT

Cotton growers face intense economic pressures as cotton prices hold steady while cost of production increases. Pathogens that cause: seed-seedling diseases, bacterial blight, Fusarium and Verticillium wilts, Phymatotrichum root rot, nematodes and leaf spots reduce yield and fiber quality. Host-plant resistance offers durable, economic control of pathogens. The multi-adversity resistance (MAR) genetic improvement program pioneered the development of cultivars resistant to several pathogens. The system utilizes specific seed, seedling, and plant screening and selection techniques in the laboratory, greenhouse and field for the simultaneous genetic improvement of resistance to pathogens, insects and abiotic stresses in addition to yield, earliness, seed and fiber quality. Since the 1970's the MAR program has developed eight MAR gene pools from which cotton cultivars and germplasm with progressive genetic improvement in resistance to pathogens, have been selected and released. Field tests are conducted each year at 10 naturally infested locations in Texas to determine the levels of resistance to pathogens. Levels of resistance to seed deterioration and seedling disease have progressed from susceptible in the MAR-1 to resistant in the MAR-8. High resistance to USA races of the bacterial blight pathogen has been maintained through the MAR gene pools. Progressive improvements in levels of resistance to root pathogens and nematodes have been made, even though no direct selection was practiced. Current MAR-8 germplasm combines higher levels of resistance to eight pathogens.

Introduction

Cotton is affected by biotic and abiotic stresses that reduce yields and fiber quality. In 1997, diseases caused an estimated 12.3% loss in yield potential representing over 2.5 million bales in the USA (Blasingame, 1998). The multi-adversity resistance (MAR) program pioneered the development of resistant cottons to several pathogens. This is a system of direct selection for a few traits and indirect genetic gains for genes and traits that provide broad resistance to adversities in cotton production along with higher yield potential, earliness, and improved quality. The MAR concepts and procedures evolved from basic research on seed-seedling cold tolerance, preservation of seed quality, and high resistance to races of the bacterial blight pathogen, and their interrelationships with resistance to several other pathogens, earliness and yield (Bird, 1982; El-Zik and Thaxton, 1989). A strong association had been shown between resistance to bacterial blight and *Fusarium* wilt/root-knot nematode complex, and a lesser but significant association between bacterial blight and resistance to *Verticillium wilt* (Bird, 1972). Selected F_4 strains are tested annually in naturally infested disease nurseries, and data are collected on disease incidence and severity for the identification of elite germplasm with higher levels of resistance to pathogens and many desired traits.

Resistance to pests is a relative plant trait rather than an absolute quality. The magnitude of resistance can range from very small to very large; plant reaction ranges from highly susceptible to immune (El-Zik and Thaxton, 1989). Resistance levels are based on comparisons with reference genotypes with known levels of resistance and susceptibility to pathogens.

Material and Methods

Maintaining genetic diversity and variability is essential for cotton improvement. The MAR system uses a short-cycle recurrent selection method for genetic improvement of cotton, using several sources of resistance to pathogens, crossed to the most advanced MAR germplasm. F_2 field selections undergo direct selection in the laboratory and greenhouse for: (1) seed coat resistance to mold; (2) slow radicle elongation after 8 days at 13.3°C; (3) cotyledon resistance to a mixture of four races of the bacterial blight pathogen (*Xanthomonas campestris* pv. *malvacearum*); and (4) absence of disease lesions at the base of the hypocotyl (resistance to *Rhizoctonia solani* and *Pythium ultimum*). Direct selection for these traits indirectly provides genetic gains for resistance to the major pathogens, insects and abiotic stresses, along with higher yield potential and earliness. Since the mid-1960's, the MAR program has created, processed and evaluated eight gene pools using the MAR procedures (Bird, 1982; El-Zik and

Thaxton, 1989; El-Zik, 1995). Elite cotton germplasm was identified and selected from each gene pool. Tests included MAR cultivars Tamcot SP37 representing the MAR-1 gene pool, Tamcot CAMD-E from MAR-2, Tamcot CD3H and Tamcot CAB-CS from MAR-4, Tamcot HQ95 from MAR-5, and Tamcot Sphinx from MAR-6, in addition to advanced strains from the MAR-7 and MAR-8 gene pools. The levels of resistance to cotton pathogens in the MAR-1 to MAR-7 have been quantified (El-Zik *et al.*, 1991; El-Zik and Thaxton, 1992; Thaxton and El-Zik, 1991; 1994; 1996; 1998).

Advanced MAR germplasm (F_5 to F_7) undergoes extensive evaluation for resistance to pathogens each year in naturally infested disease field nurseries in Texas to identify and select germplasm with resistance to several pathogens causing seed-seedling diseases (*Pythium ultimum*, *Rhizoctonia solani*), bacterial blight (*Xanthomonas campestris* pv. *malvacearum*), *Phymatotrichum* root rot (*Phymatotrichum omnivorum*), *Verticillium wilt* (*Verticillium dahliae*), *Fusarium* wilt (*Fusarium oxysporum* f. *vasinfectum*), root-knot nematode (*Meloidogyne incognita*), reniform nematode (*Rotylenchulus reniformis*), and leaf spots (*Alternaria*, *Cercospora*, and other species). The MAR tests include the Early Field Planting (EFP) Test, Uniform MAR (UMAR) Test and the Strains Test. The EFP Test consists of 32 F_5 selections from the previous year's F_4 progeny rows planted in replicated tests at six locations. The UMAR Test consists of 24 advanced MAR strains including F_6 selections from the Early Field Planting Test, grown in replicated tests at nine locations. The Strains Test includes all genotypes in the EFP Test, UMAR Test, additional elite MAR genotypes and 12 cultivar checks, and is grown in two replications at nine locations. The percentage of plants showing disease symptoms is calculated for each strain, performance ratings are made, and yield and fiber data are obtained. Each of the tests includes resistant and susceptible checks for specific pathogens.

Seed-Seedling Disease Complex. The ability of seed and seedlings to perform in cool-wet soil with minimal damage by soil fungi is a key trait of MAR cottons. Progress achieved in resistance to seed-seedling diseases was based on field stand data from the Uniform MAR Test at nine locations in 1997, and six locations in 1998. The tests consisted of 24 genotypes and four replications arranged in a randomized complete block design. One hundred acid-delinted non-graded and non-treated seeds of each genotype were planted. Seed emergence and damping-off counts were made weekly with a final stand count at 45 days. Percent seed emergence and final stand were calculated for each plot. The means for seedling disease represent cumulative losses to seed rot, pre- and post-emergence damping-off as percentage of planted seed.

Bacterial Blight. The MAR program continues to maintain high levels of resistance in its germplasm to USA races of the bacterial blight pathogen. There is intense screening and selection for resistance to the bacterial blight pathogen in the F_1 through the F_4 generations utilizing laboratory and field inoculation techniques. Methods used by the MAR program to develop cultivars and strains resistant bacterial blight, pathogen variability and sources of resistance have been reported (Thaxton and El-Zik, 1993; El-Zik and Thaxton, 1994).

Phymatotrichum Root Rot. The MAR germplasm was evaluated in the McGregor disease nursery to identify resistant strains and ascertain the levels of resistance in UMAR and Strain Tests, located in an area of known history of *Phymatotrichum* root rot with uniform inoculum density. Weekly counts of plants killed by *Phymatotrichum omnivorum* from square initiation to maturity gave a percentage of dead plants for each genotype.

Verticillium wilt. Resistance to *Verticillium wilt* is field evaluated annually at the Chillicothe and Halfway. Plants with foliar disease symptoms are counted in each plot at first flower and prior to maturity, giving a percentage of diseased plants. Prior to boll opening, a grade of one, no symptoms to five, severe defoliation, based on foliar wilt symptoms is made for each plot.

In identifying resistance levels of cotton germplasm under field conditions, the time of assessing plants with symptoms is very critical. The optimum time varies annually, based on crop progress and temperature, but usually between mid-August through September, after bolls have set (Bell, 1992; El-Zik, 1985; Thaxton and El-Zik, 1998). Counts are made every two weeks but not at harvest since the correlation between wilt severity at harvest and yield losses is poor.

Fusarium Wilt and Nematodes. *Fusarium* wilt resistance is tested at the National *Fusarium* Wilt nursery in Tallassee, Alabama. Eight advanced MAR strains including the resistant strain M-315 and the susceptible Rowden were evaluated in 1996 and 1997 for resistance to *Fusarium oxysporum* f. *vasinfectum*. Nematode resistance levels were assessed in Weslaco for reniform nematodes and at College Station for root-knot nematodes.

Analysis of variance was performed for each test and over tests and years for each disease. LSD tests determined differences among cultivars and strains.

Results and Discussion

Seed-Seedling Diseases. Adverse conditions in 1996, rain and high temperatures causing deterioration of seed produced were followed by a cold, wet spring in 1997 with a high incidence of seedling disease. Stand performance from the 1997 Uniform MAR Test

indicated significant differences among locations and strains in percentage of final stand across nine locations (Table 1) with averages ranging from 30% on the Upland Farm at College Station to 52% for the Weslaco-TAMU Test. The averaged over the nine locations ranged from 51% for SPNXCDUG8H-1-95 to 33% for CIQ2UWGPIS-1-95. Five MAR-7 gene pool strains produced field stand equal to the MAR-6 Tamcot Sphinx cultivar. The cultivar x location interaction was non-significant; thus, the mean over the nine field tests was the best measure of field stand for a given genotype.

Final stands ranged, from a low of 43% at College Station to 75% at Chillicothe in 1998with no significant differences among genotypes at Corpus Christi, Thrall and Chillicothe (Table 2). Averaged over six Uniform MAR tests, stand ranged from 59% for LBQWICQPIS-1-96 to 81% for Paymaster PM330 (fungicide-treated seed). Several new MAR-7 advanced strains gave higher field stands than Tamcot Sphinx and Deltapine 50 checks. Generally, progressive improvements in resistance to the seed-seedling pathogens, stand establishment and seedling vigour have been achieved in advanced MAR-6 to MAR-8 strains.

Bacterial Blight. Resistant cultivars offer the most economical and practical means of controlling bacterial blight. Based on field inoculation, Tamcot cultivars averaged a grade of 1.4 (highly resistant reaction) in comparison to the non-MAR cultivars grade of 5.2 (susceptible reaction). Race 18 remains the most virulent race. The MAR program maintains high levels of resistance in its germplasm to the USA races of bacterial blight.

Phymatotrichum Root Rot. The 1997 MAR Strains test at McGregor indicate significant differences among cultivars and genotypes in the percentage of plants killed by *Phymatotrichum* root rot, ranging from 5.5% for Tamcot CAMD-E to 76.5% for CBQWHG2PIS-1-96 with a mean of 32.1% (Table 3). Several MAR-7 strains show intermediate resistance, including SPNXHQBPIS-1-94, HGPIHQBPIH-2-94, CABU2HGC8H-2-91 (Tamcot Luxor) and CUBQHGRPIS-1-92 (Tamcot Lotus)

***Verticillium* Wilt.** Variability was found in MAR germplasm for resistance to *Verticillium wilt* at the Halfway nursery in 1997 (Table 4). Data abstracted from the Strains Test include 102 genotypes and commercial cultivar checks in two replications. Percentage of plants with foliar *Verticillium wilt* symptoms ranged from 25% for PD23CD3HGS-1-93 to 89% for CQPICDGP6H-1-95, and 36% for the resistant check Paymaster 330. Four MAR strains, PD23CD3HGS-1-93, OSIKRHQWIH-2-94 (okra-shaped leaf type), CUBQHGRPIS-1-92 (Tamcot Lotus) and PD22CUBQWS-1-95 were comparable to Paymaster 330 for resistance.

Fusarium Wilt

Resistance to *Fusarium* wilt in the earlier Tamcot cultivars Tamcot SP21S, Tamcot SP37H and Tamcot CAMD-E was reported (Kappelman and Bird, 1981). Three years data show that Tamcot cultivars developed by the MAR procedures had resistance levels equal to the resistant cultivar McNair 511, and were significantly less susceptible than Rowden. The development of this resistance indicates that the MAR system was effective for improving resistance to the *Fusarium* wilt/root-knot nematode complex.

Higher levels of resistance to the *Fusarium* wilt pathogen are being maintained or increased in the MAR germplasm. In the evaluation of the eight elite MAR strains in the National *Fusarium* Nursery at Tallassee, Alabama test, four strains had high levels of resistance to the *Fusarium* wilt and root-knot nematode complex, and three had intermediate levels, including the resistant check (Table 5). Two strains, HQCULHQPIH-1-95 and LGQWILBCGS-1-95, had similar resistance levels to check M-315. From 1994-1996, the MAR-7 cultivar Tamcot Luxor averaged 29.3% plants with wilt symptoms compared to 65.0% for susceptible Rowden and 6.6% for resistant M-315.

Reniform Nematodes. Results of the *reniform* nematodes in the Weslaco tests are presented. Lint yield was measured in *reniform* infested soil and Telone II treated soil. Mean lint yield over genotypes was 708 kg/ha in the untreated soil and 818 kg/ha for the Telone II treated soil (Table 6). MAR strain LGQWILBCGS-1-95 produced high and similar yield in the *reniform* and Telone treated plots, and was the third highest yielding strain in the test with 883 kg/ha lint, followed by MAR strain PD22CUBQWS-1-95. Tamcot Sphinx and Stoneville 474 produced similar yields under both treatments.

Conclusions

Germplasm from the MAR-7 and MAR-8 gene pools have higher resistance levels to pathogens causing seed-seedling disease, *Verticillium* and *Fusarium* wilts, *Phymatotrichum* root rot, leaf spots, and root-knot and reniform nematodes than previously released MAR germplasm. High resistance levels to bacterial blight have been maintained. The results of these studies indicate that genetic gains and progressive improvement in resistance to plant pathogens have been achieved in MAR germplasm from the MAR-1 to MAR-8 (Table 7). Seed deterioration and seedling disease have progressed from partial resistance and highly susceptible, respectively, in the MAR-1 to resistance/highly resistance in the MAR-8. High resistance to bacterial blight has been maintained through the MAR gene pools. Progressive improvements in resistance to the root pathogens (*Fusarium* and *Verticillium wilt*s, and *Phymatotrichum* root rot) have been made, even though no direct selection was practiced for these pathogens. Testing in

disease nurseries is essential to identify new resistant strains and ascertain the resistance in MAR germplasm.

The MAR system has proven efficient and successful in simultaneously pyramiding favourable and compatible genes for resistance to pathogens, insects and abiotic stresses. The MAR program will continue to develop and release multi-adversity resistant cotton germplasm with higher levels of resistance to cotton pathogens, insects and abiotic stresses in addition to high yield potential, earliness, and further improvement in fiber and seed quality. Improved resistance levels to pests will make it possible for growers to realize a higher proportion of the genetic potential of their cultivars, to reduce production cost and risk, and to increase profit.

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Table 1. Mean percent final stand for MAR strains and cultivars in the 1997 Uniform MAR Test at nine locations in Texas and over locations.

	Weslaco			College Station						
MAR Strain/Cultivar	USDA	TAMU	Corpus Christi	Upland Farm	Brazos Valley	Temple	McGregor	Chillicothe	Halfway	Mean / locations
SPNXCDUG8H-1-95	42*	65**	59**	46**	46**	53**	64**	32**	36**	51**
SPNXHQBPIS-1-94	59	62	52	29	42	33	52	59	40	49
PD22CDGU8S-1-95	52	59	52	33	39	41	55	57	33	49
CHGUCLG23H-1-95	46	58	49	39	49	43	49	51	31	48
PD22CUBQWS-1-95	47	55	47	39	53	47	51	37	25	47
Tamcot Sphinx check	46	57	44	31	41	39	50	58	41	46
Tamcot CAB-CS check	46	55	52	28	40	40	54	43	26	45
CIQUBCHGBS-1-95	48	57	51	30	43	39	50	39	34	45
LGQWCIQABS-1-95	38	55	51	30	38	43	50	47	39	44
HGPIHQBPIH-2-94	51	56	51	24	41	36	46	45	24	44
LGQWILBCGS-1-95	51	58	45	30	37	40	46	42	26	44
HQCULHQPIH-1-95	48	61	54	27	28	39	43	44	31	43
CUBQHGRPIS-1-92	50	50	57	23	40	38	41	44	29	43
CABU2HGC8H-2-91	41	46	47	28	42	42	46	49	38	43
CABCSV506S-1-94	40	60	49	26	39	37	36	42	37	42
CUBQHGRPIH-1-92	43	--	45	30	--	39	48	45	36	42
OSIKRHQWIH-2-94	46	54	48	27	38	39	44	35	28	41
HQCULCLBGS-1-95	40	55	47	28	39	37	45	37	25	41
SPNXCHGLBH-1-94	44	47	50	22	30	43	48	42	34	41
Paymaster PM330 check	53	50	39	26	31	31	46	49	36	41
RC5BCCUBQS-1-93	38	47	46	25	36	43	47	37	29	40
SPNXCBGP6H-1-95	49	45	46	30	33	35	33	47	40	40
CHGUA7QCUH-1-95	41	43	42	24	37	40	34	46	24	38
CIQ2UWGPIS-1-95	41	46	34	18	24	30	41	30	20	33
Mean	46	52	48	30	38	39	47	44	32	43
LSD (*P*=0.05%)†	8	11	8	11	10	8	12	9	10	8
C.V. %	17	15	11	26	26	15	15	15	21	4

The location x cultivar interaction was not significant

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

† Least significant difference between two means within column

Source: 1997 Uniform MAR Test at nine locations in Texas

Table 2. Mean final stand for MAR strains and cultivars over strains and locations (%).

Strain/Cultivar	Weslaco	Corpus Christi	Brazos Valley	Thrall	McGregor	Chillicothe	Locs.
Paymaster PM330 check	87**	87	64**	72	81**	79	81**
OHGPILBHQH-3							
SPNXCBGP6H-1							
LBCHGPI2KS-1-96	82	84	48	55	62	76	72
CIQBCHGC8H-1-96	82	74	49	71	57	81	71
LBQWICQPIS-2-96	85	75	44	59	62	71	70
CIQBCDUG8S-1-96	85	78	45	56	57	78	69
SPNXCDUG8H-1-95	87	74	43	55	58	74	69
PD22CUBQWS-1-95	82	77	43	57	57	72	68
CABU2HGC8H-2-91 check	78	76	43	61	55	75	68
SPNXCHGLBH-1-94	82	79	49	52	58	77	67
Deltapine 50 check	79	73	34	62	54	81	67
HGPIHQBPIH-2-94	82	67	53	55	53	76	64
CD2HGCILBH-1-96	77	77	40	50	54	78	64
CUBQWCILBH-1-96	80	60	43	60	56	79	64
Tamcot Sphinx check	75	74	42	59	48	79	64
HQCULHQPIH-1-95	81	69	40	49	55	75	63
CHGBCHGC8H-1-96	80	76	45	47	49	71	63
OSIKRHQWIH-2-94	75	73	32	54	49	75	63
RCBCLCQPIH-1-95	82	62	46	50	57	65	63
OHGPILBHQH-1-96	73	66	32	60	48	70	62
CIQBCDULBH-1-96	80	77	38	39	49	73	61
CUBQHGRPIS-1-92 check	81	66	45	43	53	69	61
LBQWICQPIS-1-96	72	62	23	56	44	75	59
Mean	81	74	43	56	56	75	67
LSD (*P*= 0.05)†	7	NS	11	NS	12	NS	7
C.V. %	6	18	18	28.5	15	11	17

The location x cultivar interaction was not significant

** Significant at the 0.01 probability levels.

† Least significant difference between two means within column

Source: 1998 Uniform MAR Test at six locations in Texas.

Table 7. Levels of resistance to plant pathogens in the MAR gene pools.

	Level of Resistance†				
Adversity	MAR-1	MAR-2	MAR-4	MAR-5/MAR-6	MAR-7/MAR-8
Seed deterioration	PR	IR	IR/R	R	R/HR
Seedling disease	HS	IR	IR/R	R	R/HR
Bacterial blight (US races)	HR	HR	HR	HR	HR
Fusarium wilt/root-knot	IR	R	R	R	R/HR
Nematode complex					
Verticillium wilt	PR	IR	IR	IR	R/HR
Phymatotrichum root rot	HS	PR	IR	IR	IR

† IM, immune; HR, high resistance; R, resistance; IR, intermediate resistance; PR, partial resistance; S, susceptible; HS, highly susceptible

Table 3. Plants killed by *Phymatotichum ominvorum*[1].

MAR Strain/Cultivar	Percent of plants killed	
	July 17	Aug. 14
	%	%
Paymaster HS26 check	3.3**	5.3**
Tamcot CAMD-E check	5.5	5.5
SPNXHQBPIS-1-94	4.8	8.1
HGPIHQBPIH-2-94	7.6	8.7
CABU2HGC8H-2-91	9.8	11.8
CUBQHGRPIS-1-92	9.4	14.1
:	:	:
SPNXCDUG8H-1-95	19.5	23.0
Tamcot Sphinx check	23.1	23.1
SPNXCHGLBH-1-94	23.6	23.6
:	:	:
PD22CUBQWS-1-95	47.8	61.3
LBCHGPI2KS-1-96	49.8	63.9
LBQWICQPIS-2-96	63.2	65.5
CBQWHG2PIS-1-96	73.2	76.5
Mean	28.1	32.1
LSD (*P*= 0.05)†	42.0	38.0

** Significant at the 0.01 probability level
† LSD difference between means within column
Source: 1997 MAR Strains Tests at McGregor, Texas.

Table 4. Plants with *Verticillium* wilt leaf symptoms[1].

Strain/cultivar	Percent Foliar Wilt	
	Aug. 30	Sept. 24
MAR-PD23CD3HGS-1-93	12**	25**
MAR-OSIKRHQWIH-2-94	13	32
MAR-CUBQHGRPIS-1-92	23	32
Paymaster 330 check	12	36
MAR-PD22CUBQWS-1-95	14	37
:	:	:
Tamcot Sphinx check	20	51
Deltapine 50 check	19	53
:	:	:
Stoneville 887 check	10	65
:	:	:
MAR-CQPICDGP6H-1-95	22	89
Test Mean	20	49
LSD (*P*= 0.05)†	12	35

** Significant at the 0.01 probability level.
Source: 1997 MAR Strains Test at Halfway, Texas

[1] Data abstracted from test that included 102 genotypes and commercial cultivar checks.

Table 5. Percent plants with *Fusarium* wilt symptoms.

Strain/cultivar	Percent wilt
M-315 Resistant check	21
MAR-HQCULHQPIH-1-95	24
MAR-LGQWILBCGS-1-95	31
MAR-SPNXHQBPIS-1-94	39
MAR-SPNXCHGLBH-1-94	40
MAR-HGPIHQBPIH-2-94	48
MAR-CABCSV506S-1-94	57
MAR-SPNXCDUG8H-1-95	58
MAR-CIQUBCHGBS-1-95	71
Rowden Susceptible check	89
Mean	47.5

Source: 1997 National *Fusarium* Nursery, Tallassee, Alabama.

Table 6. Lint yield of cotton strains and cultivars (kg/ha)[2].

MAR Strain/cultivar	Lint Yield		
	Reniform	Telone II	Mean
	kg/ha	kg/ha	kg/ha
MAR-LGQWILBCGS-1-95	883**	881**	883**
MAR-PD22CUBQWS-1-95	784	961	872
MAR-CABCSV506S-1-94	722	797	759
Deltapine 5409	559	811	684
Tamcot Sphinx	651	668	657
Stoneville 474	641	646	643
Stoneville LA 887	458	708	582
Test Mean	708	818	763
LSD (P=0.05)†	200	234	153

** Significant at the 0.01 probability level
† LSD between two means within a column
Source: Reniform nematode test in 1997 at Weslaco, Texas

[2] Data abstracted from a 24 entry test Conducted by Dr. C.C. Cook, USDA-ARS, Weslaco, Texas