THRIPS RESISTANCE IN COTTON: GERMPLASM EVALUATION, INHERITANCE AND QTL MAPPING

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Abstract

Thrips is one of the most important insect pests in early cotton-growing season in the United States and causes yield loss up to 1% Beltwide after one insecticide application. Developing and growing thrips resistant cultivars represents the most economic strategy in thrips management. A project was initiated to study the genetic basis of thrips resistance in Upland (*Gossypium hirsutum*) x Pima (*G. barbadense*) crosses and to identify thrips resistant lines from the interspecific hybrids. The major results are as the following: (1) Among the five tetraploid cotton species, *G. tomentosum* with the Pilose trait was the most resistant, followed by *G. mustelinum*, *G. barbadense* and *G. darwinii*, and Upland cotton was the most susceptible. (2) In 14 F_2 populations of four Pima x four Upland crosses, thrips resistance in four populations showed a 3 resistant: 1 susceptible ratio, indicating a major thrips resistance gene. (3) Among 146 backcross inbred lines (BILs) derived from Upland SG 474 x Pima S-7 and 90 recombinant inbred lines (RILs) derived from Acala 1517-99 x Pima Phy 76, more than 30 lines displayed similar thrips resistance to the Pima cotton parents, indicating that the thrips resistance in Pima cotton was successfully transferred into Upland cotton through backcrossing or pedigree selections. (4) Broad-sense heritability estimates for thrips resistance ranged from 0.68 to 0.79, indicating that the majority of the variation in thrips resistance is determined by genetic factors. The results in both Mendelian and quantitative genetics are consistent and corroborated with the efficient transfer of the thrips resistance from Pima to Upland cotton. Mapping of the major thrips resistance gene and other quantitative trait loci (QTLs) is ongoing using the BIL population with more than 500 molecular markers.

Introduction

Thrips (*Thrips* and *Frankliniella* spp.) is one of the most important insect pest challenges in early cotton-growing season in the United States and many other cotton-producing countries. Its infestation on apical meristems and young leaves of cotton seedlings causes distortion, malformation and tearing of leaves, reduction of leaf area, plant height and root growth, or death of apical meristems, which can lead to excessive vegetative branching, delay in fruit setting and plant maturity, and reduced yield. From 1986 to 2009, 56 to 96% of the cotton acreage in the U.S. was infested with thrips during the seedling growth stage each year. Even though insecticide applications for thrips were applied 0.19 to 1.1 times per acre, the Beltwide yield losses ranged from 0.12 to 0.88% (Cook et al., 2011).

Genetic variation in thrips resistance among cotton species and genotypes was long noted (Ballard, 1951; Hawkins et al., 1966; Rummel and Quisenberry, 1979; Quisenberry and Rummel, 1979) and screening a large number of germplasm accessions for thrips resistance was also reported (Arnold et al., 2012; Stanton et al., 1992). Upland cotton (*Gossypium hirsutum*) is in general highly susceptible to thrips and breeding cotton for thrips resistance is one of the objectives in several cotton breeding programs in the U.S., resulting in releases of cultivars and breeding lines with moderate levels of thrips resistance (e.g., Bourland and Jones, 2005; Thaxton and El-Zik, 2004).

Over the years, we have observed that Pima cotton is more resistant to thrips in breeding nurseries where both Pima and Upland cotton were grown. This phenomenon was also reported by others (e.g., Bowman and McCarty, 1997). However, the genetic basis of thrips resistance in Pima cotton is currently poor understood (Bowman and McCarty, 1997). At New Mexico State University, a project was initiated to understand the genetic basis of thrips resistance in

Pima cotton and to identify advanced breeding lines for thrips resistance in an advanced backcross inbred line (BIL) population and a recombinant inbred line (RIL) population derived from crosses between Upland and Pima cotton.

Materials and Methods

Plant materials

Cotton species: In one test, five Upland cotton (*G. hirsutum*, AD1) cultivars or lines and eight Pima cotton (*G. barbadense*, AD2) cultivars or lines were grown in Jan. 2012 in the greenhouse for thrips evaluation. In another test, 10 *G. barbadense* (AD2) cultivars from China and Egypt were also planted in Jan. 2012 in the greenhouse and evaluated for thrips damage on March 24, 2012. For a comparison purpose, *G. tomentosum* (AD3), *G. mustelinum* (AD4) and *G. darwinii* (AD5) each represented by 2-6 accessions, were also evaluated in the greenhouse.

Upland x Pima F_2 populations: Four elite Upland cotton genotypes, Acala 1517-08 (Zhang et al., 2011), LA 35RS, MD25-27Y and UA 48 (Bourland and Jones, 2011) were used as males to cross with four Pima cotton cultivars, Cobalt, DP 340, Pima S-7 and Phy 830. The resulting F_1 hybrids except for DP 340 x UA 48 and Phy 830 x LA 35RS were selfed to produce F_2 populations. The 14 F_2 populations and their parental lines were planted in the greenhouse in mid-May 2012 and evaluated for thrips resistance in early July 2012.

A backcross inbred line (BIL) population: 146 BILs, which were derived from a cross of Upland cotton SG 747 x Pima S-7 followed by two generations of backcrossing and three generations of selfing, together with the two parents and Acala 1517-99 (Cantrell et al., 2000), were grown in the field, Las Cruces, NM, in early May 2008 . The experiment was arranged in a randomized complete block design with three replicates (1-row x 30 ft long plots). Thrips damage in each plot was evaluated on June 13, 2008.

Advanced breeding lines: 90 lines from a repeated pedigree selection process in a cross of Acala 1517-99 x Pima Phy 76 were divided into three different tests, each of which had 30 lines, two parents and Acala 1517-08. Seed was sown in the greenhouse in Jan. 2012 and seedlings were evaluated for thrips resistance on Mar. 24, 2012.

Evaluation of thrips resistance

A rating scale of 0 to 7 was used to rate individual plants or plots for thrips resistance in the field or greenhouse:

0	no symptom
1	very light symptom, very small mottled dots on leaves
2	light symptom, small mottled appearance of leaf, no wrinkled leaf
3	moderate symptom, malformation and tearing of leaf
4	severe symptom, injury of apical meristem
5	very severe symptom, death of apical meristem and severely wrinkled leaf
6	nearly dead, death of apical meristem and defoliation
7	dead plant

Data analysis

Analyses of variance (ANOVA) were performed (SAS, 2000) for separation of means using the least square difference (LSD) and broad-sense heritabilities were estimated based on ANOVA.

PCR and DNA markers

PCR-based markers including amplified fragment polymorphism (AFLP), simple sequence repeats (SSR), sequence tagged sites (STS) and single strand conformation polymorphism (SSCP) were developed for the BIL population.

Results and Discussion

Thrips resistance in five tetraploid cotton species

As expected (Table 1), the five Upland cotton genotypes (with ratings of 1.8 to 3.1) were more sensitive to thrips damage than the eight Pima cottons (with ratings of 1.0 to 2.7), five of which had ratings below 1.6. Due to the hairy leaves in *G. tomentosum*, no visible thrips damage was observed (Table 2). Pilose cotton was long recognized to confer resistance to thrips (Bowman and McCarty, 1997; Walker et al., 1979). Other two wild tetraploid species *G. mustelinum* and *G. darwinii* were also resistant to thrips infestations. The 10 exotic *G. barbadense* cultivars (7 from China and 3 from Egypt) were consistently resistant to thrips with average ratings ranging from 1 to 2 (Table 2).

Table 1. Average ratings of thrips responses in two cultivated tetraploid cotton species.

Species	Line	Rating
Gossypium hirsutum (AD1)	Acala 1517-08	2.1
Gossypium hirsutum (AD1)	UA 48	2.1
Gossypium hirsutum (AD1)	MD 25-27Y	2.3
Gossypium hirsutum (AD1)	DP 393	3.1
Gossypium hirsutum (AD1)	LA S35RS	1.8
Gossypium barbadense (AD2)	Pima S-7	1.6
Gossypium barbadense (AD2)	Phy 830	1.5
Gossypium barbadense (AD2)	Cobalt	2.1
Gossypium barbadense (AD2)	DP 340	1.5
Gossypium barbadense (AD2)	06E2032-11	1.2
Gossypium barbadense (AD2)	Pima 32	1.3
Gossypium barbadense (AD2)	Monseratt SI	2.7
Gossypium barbadense (AD2)	11NM15-Giza	1.1

Table 2. Average ratings of thrips responses in four tetraploid cotton species.

Species	Line	Rating
Gossypium barbadense (AD2)	Dandara	2.0
Gossypium barbadense (AD2)	Giza 70	1.3
Gossypium barbadense (AD2)	Giza 83	1.7
Gossypium barbadense (AD2)	Xinhai 16	2.0
Gossypium barbadense (AD2)	Xinhai 20	2.0
Gossypium barbadense (AD2)	Xinhai 24	1.6
Gossypium barbadense (AD2)	Xinhai 25	1.0
Gossypium barbadense (AD2)	Xinhai 30	1.3
Gossypium barbadense (AD2)	Xinhai 35	1.6
Gossypium barbadense (AD2)	Xinhai 36	1.6
Gossypium tomentosum (AD3)		0.0
Gossypium mustelinum (AD4)		1.7
Gossypium darwinii (AD5)		1.0

Segregation analysis of thrips resistance in Upland x Pima F2 populations

Of all 16 possible F_2 populations (except for DP 340 x UA 48 and Phy 830 x LA 35RS) between four Pima and four Upland cotton genotypes, seedling responses to thrips damages in four interspecific crosses followed an 1 R resistant (rating below or equal to 2) : 1 susceptible (rating higher than 2) ratio (Table 3). The results clearly indicate that Pima cotton carries one major resistance gene to thrips. In most of other crosses, escapes from thrips injury may have inflated the numbers of resistant plants, resulting in distorted segregation deviating from the expected 3:1 ratio.

Table 3. Segregation ratio of thrips resistance in 14 Upland x Pima F_2 populations.

Cross	No. plants with different ratings											No.	No.	Exp.	χ^2
	0	1	1.5	2	2.5	3	3.5	4	5	6	7	R	S	ratio	
Cobalt x 1517-08	2	85	0	15	0	5	0	0	0	0	0	87	15	3R: 1S	5.76
Cobalt x LA 35RS	5	75	0	4	0	1	0	0	0	0	0	80	5	3R: 1S	16.57
Cobalt x MD 25-27Y	2	53	0	24	0	12	0	1	0	0	0	79	13	3R: 1S	5.80
Cobalt x UA 48	2	49	0	22	0	13	0	2	0	0	0	73	15	3R: 1S	2.97
DP 340 x 1517-08	3	42	0	29	0	23	0	2	2	0	0	74	27	3R: 1S	0.16
DP340 x LA 35RS	0	27	2	24	11	14	3	6	0	0	0	53	34	3R: 1S	9.20
DP 340 x MD 25-27Y	0	52	0	24	0	9	0	3	0	0	0	76	12	3R: 1S	6.06
Phy 830 x 1517-08	1	48	54	20	2	3	0	1	0	0	0	74	6	3R: 1S	13.07
Phy 830 x MD 25-27Y	0	48	0	27	0	8	0	2	1	0	0	75	11	3R: 1S	6.84
Phy 830 x UA 48	1	53	0	26	0	6	0	1	0	0	0	80	7	3R: 1S	13.34
Pima S-7 x 1517-08	1	21	0	30	0	14	0	10	0	0	0	52	24	3R: 1S	1.75
Pima S-7 x LA 35RS	1	53	6	15	7	2	0	0	0	0	0	75	9	3R: 1S	9.14
Pima S-7 x MD 25-27Y	0	46	0	22	0	8	0	4	0	0	2	68	14	3R: 1S	2.75
Pima S-7 x UA 48	0	16	0	46	0	29	0	3	0	0	0	62	32	3R: 1S	4.10

$\chi^2_{(0.05\ df=1)} = 3.84$.

Evaluation of thrips resistance in BILs derived from Upland x Pima

The success of transfer of thrips resistance in Pima cotton was evaluated in a backcross inbred line (BIL) population of 146 lines in a replicated field test (Table 4). The Upland cotton parent SG 747 was more sensitive to thrips than Pima S-7, while Acala 1517-99 (with an average rating of 3) was intermediate in response to thrips damage. It appeared that more than 10 BILs were as resistant as the Pima parent to thrips, a proportion as expected based on one major resistance gene in the Pima parent from the BIL population derived from two backcrosses and three selfings. The results indicate that the thrips resistance in Pima cotton was transferred into Upland cotton through backcrossing without selection for thrips resistance during the breeding process.

Table 4. Thrips resistance in selected backcross inbred lines derived from SG 747 x Pima S-7.

Most tolerant		Most susceptible	
Line	Rating	Line	Rating
NMHT-61	2.50	NMHT-17	4.83
NMHT-56	2.67	NMHT-71	4.83
NMHT-27	2.83	NMHT-97	4.83
NMHT-38	2.83	NMHT-104	5.00
NMHT-100	3.00	NMHT-70	5.00
NMHT-115	3.00	NMHT-85	5.00
NMHT-116	3.00	NMHT-93	5.00
NMHT-60	3.00	NMHT-128	5.17
NMHT-73	3.00	NMHT-133	5.17
NMHT-80	3.00	NMHT-19	5.17
NMHT-88	3.00	NMHT-123	5.50
NMHT-95	3.00	NMHT-126	5.50
Pima S-7 (P1)	2.67	SG 747 (P2)	4.67

Evaluation of thrips resistance in advanced breeding lines derived from Upland x Pima

The success of transfer of thrips resistance in Pima cotton was further evaluated in advanced breeding lines selected for field agronomic performance from the cross of Acala 1517-99 x Pima Phy 76 (Table 5). A total of 21 lines had similar thrips responses to the Pima cotton parent, indicating that the thrips resistance in Pima cotton was indeed transferred into Upland cotton through a long term pedigree selection process.

Heritabilities of thrips resistance in Upland x Pima

Since the analysis of variance showed significant genotypic variation in thrips responses within each of the three greenhouse tests (Table 5), heritabilities for thrips resistance were estimated to be from 0.68 for Test 2 and 3 to 0.79 for Test 1. The results indicates that thrips resistance in the breeding lines derived from Upland x Pima was moderately high, implying that phenotypic selection for thrips resistance is efficient through replicated tests. After evaluating hybrids from five *G. barbadense* genotypes x four Upland cultivars and 90 converted racestocks for tolerance to thrips, Bowman and McCarty (1997) reported that general combining ability was significant for thrips damage ratings in the F_1 generation among the *G. barbadense* parents, while specific combining ability was detected in F_2, and concluded that thrips resistance was mainly determined by the non-additive genetic variance.

Table 5. Thrips resistance in advanced breeding lines derived from Acala 1517-99 x Pima Phy 76.

Test 1			Test 2			Test 3		
Line	**ID**	**Rating**	**Line**	**ID**	**Rating**	**Line**	**ID**	**Rating**
08N1141	10AYT05	**2.21**	08N1635	10AYT50	**2.23**	08N1835	10AYT87	**2.20**
08N1196	10AYT09	**2.50**	08N1595	10AYT36	**2.64**	08N1770	10AYT74	**2.32**
08N1186	10AYT07	**2.60**	08N1653	10AYT52	**2.67**	08N1782	10AYT76	**2.33**
08N1198	10AYT10	**2.63**	08N1589	10AYT33	**2.69**	08N1747	10AYT70	**2.50**
08N1514	10AYT25	**2.66**	08N1599	10AYT37	**2.80**	08N1740	10AYT67	**2.51**
08N1220	10AYT13	**2.67**	08N1592	10AYT35	2.87	08N1773	10AYT75	**2.73**
08N1367	10AYT23	2.75	08N1619	10AYT43	2.88	08N1817	10AYT84	**2.77**
08N1527	10AYT27	2.78	08N1586	10AYT32	2.93	08N1735	10AYT64	**2.80**
08N1184	10AYT06	2.80	08N1563	10AYT46	2.97	08N1547	10AYT30	**2.83**
08N1210	10AYT12	3.00	08N1579	10AYT48	2.97	08N1762	10AYT73	**2.83**
08N1320	10AYT21	3.02	08N1702	10AYT56	2.97	08N1724	10AYT63	2.91
08N1046	10AYT02	3.03	08N1614	10AYT40	3.06	08N1749	10AYT71	2.91
08N1084	10AYT03	3.09	08N1602	10AYT38	3.23	08N1825	10AYT86	2.91
08N1518	10AYT26	3.19	08N1704	10AYT58	3.27	08N1736	10AYT65	3.07
08N1206	10AYT11	3.22	08N1590	10AYT34	3.30	08N1786	10AYT77	3.10
08N1240	10AYT15	3.32	08N1562	10AYT45	3.31	08N1742	10AYT68	3.20
08N1064	10AYT04	3.34	08N1699	10AYT55	3.32	08N1792	10AYT80	3.20
08N1303	10AYT20	3.38	08N1716	10AYT59	3.36	08N1717	10AYT60	3.23
08N1537	10AYT29	3.38	08N1698	10AYT54	3.43	08N1787	10AYT78	3.23
08N1325	10AYT22	3.40	08N1615	10AYT41	3.43	08N1739	10AYT66	3.25
08N1530	10AYT28	3.46	08N1636	10AYT51	3.51	08N1789	10AYT79	3.25
08N1020	10AYT01	3.47	08N1703	10AYT57	3.51	08N1718	10AYT61	3.47
08N1503	10AYT24	3.47	03N1155	10AYT49	3.54	08N1722	10AYT62	3.50
08N1302	10AYT19	3.53	08N1559	10AYT31	3.58	08N1810	10AYT83	3.53
08N1190	10AYT08	3.64	08N1564	10AYT47	3.58	08N1823	10AYT85	3.58
08N1256	10AYT18	3.67	08N1685	10AYT53	3.60	08N1745	10AYT69	3.59
08N1222	10AYT14	3.68	08N1618	10AYT42	3.61	08N1805	10AYT82	3.61
08N1254	10AYT16	4.00	08N1603	10AYT39	3.72	08N1803	10AYT81	3.67
08N1255	10AYT17	4.07	08N1633	10AYT44	3.78	08N1755	10AYT72	3.73
1517-99	CK1	3.49	1517-99	CK1	3.36	1517-99	CK1	3.02
Pima Phy 76	CK2	1.42	Pima Phy 76	CK2	1.77	Pima Phy 76	CK2	1.75
1517-08	CK3	3.48	1517-08	CK3	3.28	1517-08	CK3	3.47
MSE		0.88	MSE		0.60	MSE		0.69
F		3.70	F		2.13	F		2.10
LSD (0.05)		1.28	LSD (0.05)		1.05	LSD (0.05)		1.13

$F_{0.05,\ df1=31/df2=62}$ =1.64. Means in bold were not significantly different from that of Pima Phy 76 at the 0.05 probability level.

Quantitative trait locus (QTL) mapping for thrips resistance

More than 500 molecular markers were developed for the BIL population and QTL analysis for thrips resistance is currently underway.

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