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BREEDING, GENETICS, & GENOMICS

Evaluation of Cotton Cultivars and Breeding Lines for Tolerance to Monosodium Methanearsonate (MSMA) Under Field Conditions

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ABSTRACT

Monosodium methanearsonate (MSMA) is an organic arsenical herbicide used to control weeds such as grasses and nutsedges in Upland cotton (*Gossypium hirsutum*) production. Transient crop injury, yield reduction, and maturity delays have been observed in commercial Upland cotton. It is unknown if genetic variation in MSMA tolerance in cotton exists. In this field study, seven replicated tests were conducted in the same field to compare MSMA tolerance among 212 commercial cotton cultivars and advanced breeding lines. The tests were sprayed over the top at the 4-true-leaf stage, and seedlings were assessed for crop injury severity on a scale of 0 (no injury) to 5 (death). Significant genotypic variation in MSMA tolerance was detected in three tests, and broad-sense heritability estimates for MSMA tolerance ranged from 0.476 to 0.846 with a mean of 0.712, indicating that most phenotypic variation in MSMA tolerance is heritable. Nine tested *G. barbadense* genotypes, including seven commercial Pima cultivars and two Sea-Island cotton lines, exhibited minimal crop injury with severity ratings of 0.40 to 0.83 (except for one cultivar with 1.33). Among the remaining 203 Upland cotton genotypes with crop injury ratings ranging from 0.90 to 3.67, five commercial transgenic cultivars and 38 public breeding lines exhibited various levels of MSMA tolerance. The results represent the first study in germplasm evaluation for MSMA tolerance and identify a set of tolerant cotton genotypes that can be selected in cotton production or used to develop new cultivars for commercial cotton production.

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Monosodium methanearsonate (MSMA), i.e., methylarsinic acid (CH_4AsNaO_3), is an organic arsenical herbicide used to control weeds such as grasses and sedges in cotton (*Gossypium hirsutum* L.) production (Kleifeld, 1970), and has been tested recently as a layby application component to control Palmer amaranth (*Amaranthus palmeri* S. Wats) (Hand et al., 2021; Price et al., 2021). Organic arsenicals are used on approximately half of the cotton acreage in the U.S. (Frans, 1972). MSMA is registered as a postemergence herbicide and mostly used in mixtures with other herbicides for emerged weeds in cotton from 3 to 4 inches tall until before the first bloom for a maximum of two topical applications (Bridges et al., 2002; Corbett et al., 2002; Culpepper et al., 2004; Frans, 1972; Thomas et al., 2006). Although the exact mode of action in weed control is currently unknown, MSMA could be involved in cell membrane destruction, cell division, and inhibition of photosynthesis and respiration through acting as an uncoupler of mitochondrial oxidative phosphorylation to interfere with ATP production in weeds (Dayan and Watson, 2011). Once absorbed by plants, MSMA can combine with sugars, amino acids, and other organic acids, and is converted to inorganic arsenic forms through demethylation (Mahoney, 2014). The herbicide is strongly adsorbed to soil particles and has low to medium mobility and little leaching, with an average half-life of approximately 240 d in non-irrigated soils and 55 d in irrigated soils (Gao and Burau, 1997; South et al., 2007). MSMA degradation in soil is primarily through oxidative demethylation by microorganisms (Akkari et al., 1986). Because its uptake by roots is limited, the primary pathway into plants is through leaves by spray applications, causing leaf cell membrane destruction and rapid desiccation of treated weeds. Movement out of treated leaves, including cotyledons, into untreated leaves of cotton was low (Keeley and Thullen, 1971). MSMA is classified as an acutely toxic substance, and there have been concerns about its potential adverse effects on the environment and human health (Gannon and Polizzotto, 2016).

Since the 1970s, the effects of MSMA on growth, yield, yield components, and fiber quality of cotton have been extensively investigated. As with other herbicides, the effects of MSMA on cotton are variable and depend on cotton growth stage, rate and type of application, and environmental factors. For example, Keeley and Thullen (1971) showed that crop injury in cotton was dependent on temperature in that cotton exposed at 31 °C at the first true-leaf stage tolerated MSMA, whereas cotton plants exposed at 13 and 20 °C were severely injured. Visible crop injury of cotton due to MSMA includes stunting, leaf purpling, and stem reddening (Culpepper et al., 2004; Kleifeld and Sachs, 1973; Snipes and Byrd, 1994). However, early season cotton injury and discoloration are transient and can be minimal, and treated cotton plants usually recover within 4 to 6 wk after treatment (Burke et al., 2004; Clewis et al., 2008; Hamilton and Arle, 1970). As a result, cotton yield and fiber quality might not be affected negatively when MSMA is applied topically early in the seedling stage (Allen et al., 1997; Baker et al., 1985; Burke et al., 2004; Hamilton and Arle, 1970; Kleifeld and Sachs, 1973; Oakley et al., 1983). However, Snipes and Byrd (1994) observed significant cotton yield reduction when MSMA was applied at the cotyledon to 1-leaf stage. The most consistent deleterious effect due to topical application of MSMA on plants taller than 6 inches, at the pinhead square stage, or 12-leaf stage is the reduction in cotton yield and delay in growth and maturity in most field studies (Allen et al., 1997; Arle and Hamilton, 1971, 1976; Edenfield et al., 2005; Frans et al., 1988; Jeffrey et al., 1972; Monks et al., 1999; Oakley et al., 1983). Other consistent adverse effects include reduction in plant height, plant internode length, boll weight, and number of fruiting branches, flowers, and mature bolls especially on the first and second fruiting positions (Arle and Hamilton, 1971; Frans et al., 1988; Oakley et al., 1983; Shankle et al., 1996; Snipes and Byrd, 1994). However, fiber quality traits usually are not affected (Arle and Hamilton, 1971, 1976; Hamilton and Arle, 1970; Snipes and Byrd, 1994). Through a multi-year study, Arle and Hamilton (1971) compared single overall applications of MSMA at 2.2, 4.5, 6.7, and 9 kg ha^{-1} applied 2, 4, 6, or 8 wk after emergence and showed that cotton yields were reduced by single applications of MSMA at the later dates and higher rates, and repeated applications of MSMA at 2.2 and 6.7 kg ha^{-1} at 2-wk intervals further decreased yields, boll weight, lint percentage, and seed number boll^{-1}, and increased fiber fineness. High concentrations or consecutive overhead applications of MSMA exact more crop injury and cause greater yield losses (Arle and Hamilton, 1976; Frans et al., 1988; Kleifeld and Sachs, 1973; Monks et al., 1999). Additionally, foliar applications of MSMA can reduce populations of certain insects such as *Pseudatomoscelis seriatus* (Reuter), *Frankliniella* spp., *Orius insidiosus* (Say), *Nabis* spp., *Lygus lineolaris* (Palisot de Beauvois), and Cicadellidae species (Baker et al., 1985; Stam, 1978).

Not all traits in cotton are affected by topical applications of MSMA. Through field tests in Georgia and Alabama, Monks et al. (1999) showed that MSMA applied postemergence in cotton at the pinhead square stage had no effect on height, node ratio, reproductive or vegetative node production, or square retention at the first or second fruiting position; and cotton maturity response to MSMA ranged from no effect to delayed maturity. Several studies have shown that directed applications of MSMA can cause crop injury and adversely affect crop development in early-stage cotton plants, but did not affect cotton yields, yield component traits, and fiber quality (Ferrell et al., 2007; Hamilton and Arle, 1970; Kleifeld and Sachs, 1973; Kleifeld, 1973). It is noted that most, if not all, of the studies used one or two commercial Upland cotton cultivars. Whether different cotton species or germplasm lines respond to MSMA differently is currently unknown.

The objectives of this study were to compare field responses of a total of 224 cotton entries representing 212 commercial Upland (*G. hirsutum*) and Pima (*G. barbadense* L.) cultivars and advanced breeding lines to a topical application of MSMA at the 4-true-leaf stage. Tolerant cultivars and elite lines were identified in cotton for the first time.

MATERIALS AND METHODS

Materials and Experimental Designs. This field study was part of field tests in the New Mexico cotton breeding program, including an annual Official Variety Test (OVT), an annual Regional High Quality (RHQ) test, and an annual Regional Breeder's Testing Network (RBTN) test, in addition to several Advanced Yield Tests (AYT) for lines developed by the program. Specifically, the current study consisted of seven different tests, designated 21OV, 21HQ, 21RB, 21P, 21R, 21S, and 21T each with 32 entries, and were performed in the same field of the cotton breeding nursery, Leyendecker Plant Science Center, New Mexico State University (NMSU), near Las Cruces, NM. Test 21OV referred to OVT with 17 commercial transgenic cotton cultivars including 11 Upland and 6 Pima cultivars from seed companies and 15 advanced breeding lines (13 Upland and 2 Pima) from the NMSU cotton breeding program. Test 21HQ referred to RHQ test and involved 18 commercial transgenic cotton cultivars from seed companies (14 Upland and 4 Pima) and 14 advanced breeding lines from four U.S. public breeding programs. For both 21OV and 21HQ, Pima PHY 881 RF and Upland DP 1948 B3XF were used as checks. Test 21RB referred to RBTN and involved 25 non-transgenic lines from eight U.S. public cotton breeding programs including NMSU, together with four non-transgenic commercial checks (DP 393, DP 493, FM 958, and UA 222) and three lines from a private seed company. Tests 21P, 21R, 21S, and 21T were AYT for breeding lines developed in the New Mexico cotton breeding program, where Acala 1517-08 (released by the same program, Zhang et al., 2011) was used as a check. The developer or source information for each cultivar or line can be found within the tables.

Each test was arranged in a randomized complete block design with three (21P, 21R, 21S, and 21T) or four replications (21OV, 21 HQ, and 21RB). On 20 May 2021, seeds (at the seeding rate of 10 seed m^{-1}) for the above seven tests were mechanically planted in one row (21P, 21R, 21S, and 21T) or two rows (21RB, 21 HQ, and 21OV) that were 10 m long (one plot for each genotype in each replication) using a four-row plot planter. The row spacing was 1.0 m. Fields were furrow irrigated immediately after planting to achieve a uniform germination and seedling stand, which was followed by another furrow irrigation event in June. Other crop management practices followed local recommendations for cotton production, aside from no insecticide being applied during the production season.

Crop Injury in Response to MSMA and Data Analysis. Because of widespread nutsedge infestation in the experimental field, the field was sprayed over the top with Target 6 Plus (Luxemburg Pamol Inc., Houston, TX), which contains 48.3% MSMA as the active ingredient, at a recommended rate of 40 fl oz ac^{-1} on 10 June 2021 (i.e., 21 d after planting, DAP), when the seedlings from the seven tests were at the 4-true-leaf stage. After plant responses to MSMA were observed daily, a final assessment for crop injury due to MSMA was conducted on 17 June 2021 (i.e., 7 d after treatment, DAT). At this time, the nutsedge was effectively controlled (Fig. 1). For each plot (10 plants were randomly sampled), a severity rating was given based on the overall severity of crop injury on a scale of 0 to 5 (0 = no apparent injury; 5 = seedling death) (Fig. 2). For each test, data from only three replications were collected. A least significant difference (LSD) at $p < 0.05$ was used to compare genotypes, following an analysis of variance (ANOVA). Broad-sense heritability (Hb) for the response to MSMA was estimated on a genotypic mean basis for each test with a significant genotypic variation based on ANOVA, as: Hb = (MSG-MSE)/MSG, where MSG is the mean square for genotype, and MSE is the mean square for experimental error.

Figure 1. The growth of nutsedges was suppressed 7 days after treatment of MSMA. Left, MSMA-treated nutsedge. Right, untreated nutsedge.

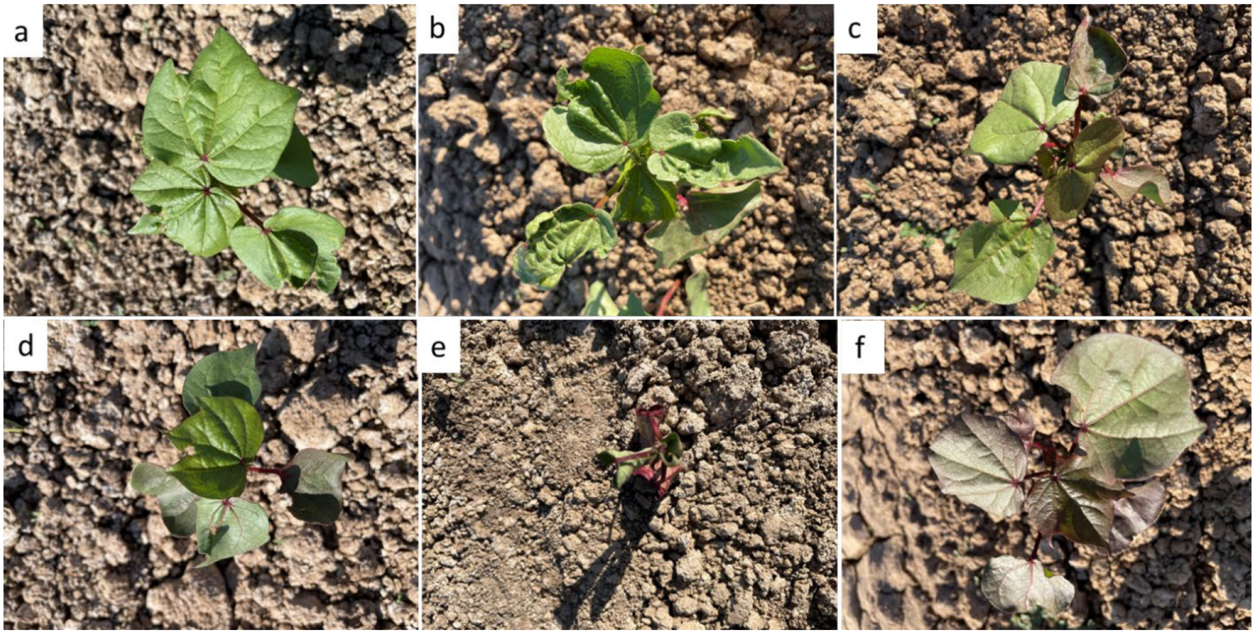

Figure 2. A rating scale based on crop injury severity caused by topical application of MSMA: a, rating 0, no injury; b, rating 1, purpling of cotyledons; c, rating 2, purpling of 1-2 true leaves; d, rating 3-4, all leaves purpling; e, rating 5, seedling death; and f, a genetic mutant with red plants.

RESULTS AND ANALYSIS

Analysis of Variance (ANOVA) and Broad-Sense Heritability of Cotton Tolerance to MSMA. The ANOVA (Table 1) detected significant genotypic variation ($p < 0.05$ or $p < 0.01$) in tolerance to MSMA in the 21OV, 21HQ, and 21RB tests. In addition, replication effects were also detected in each of the three tests and one AYT test, indicating the effectiveness of blocking control used in the experimental design. Because all genotypes tested in these three tests were unknown for their response to MSMA with various pedigrees from different breeding programs or seed companies, the choosing of the genotypes in each of the three tests can be considered random samples. The Hb in MSMA tolerance was estimated (Table 1). The Hb estimates ranged from 0.476 to 0.846 with an average of 0.712, indicating that most phenotypic variation in MSMA tolerance in cotton was due to genetic factors. The results suggest that MSMA tolerance in cotton is a moderately heritable trait. However, there was no significant genotypic difference in four AYT (21P, 21R, 21S, and 21T) for Upland cotton lines developed in the New Mexico cotton breeding program, due to similar pedigrees for these lines developed from the same New Mexico cotton breeding program.

MSMA Tolerance in Commercial Pima and Upland Cotton Cultivars. For Test 21OV (Table 2), results showed that all six Pima genotypes had the lowest crop injury ratings (mean 0.79, range 0.40-1.33), indicating their tolerance to MSMA as a cultivated tetraploid species. In comparison, Upland cotton, another tetraploid species, was sensitive to MSMA with a significantly higher average crop injury rating (1.85) than the mean crop injury for Pima cotton based on an orthogonal t test. However, the 26 Upland cotton genotypes had ratings ranging between 0.83 and 3.67 and exhibited significant differences within them. Among 11 commercial Upland cotton cultivars, FM 2498GLT had the lowest crop injury rating (1.33).

Once again, the four Pima cultivars tested had the lowest crop injury ratings (0.50-0.83) with a mean of 0.72, which is significantly lower than the mean value for Upland cotton (1.80) in Test 21HQ (Table 3). However, as compared to the Pima cotton mean, four Upland cultivars (DP 1646 B2XF, PHY 332 W3FE, PHY 390 W3FE, and PHY 764 WRF) had similar crop injuries (1.17-1.50).

Table 1. Analysis of variance of cotton tolerance to MSMA in seven replicated field tests, Las Cruces, NM, June 2021

Test	Source of Variation	df	MS	F	Hb[z]
21OV	Rep	2	0.99	3.22	0.846
	Genotype	31	1.99	6.49[y]	
	Error	62	0.31		
21HQ	Rep	2	1.00	3.99	0.815
	Genotype	31	1.35	5.40[y]	
	Error	62	0.25		
21RB	Rep	2	4.03	12.03	0.476
	Genotype	31	0.64	1.91[x]	
	Error	62	0.34		
21P	Rep	2	0.64	3.06	ns[w]
	Genotype	31	0.33	1.55	
	Error	62	0.21		
21R	Rep	2	1.38	6.62	ns
	Genotype	31	0.33	1.58	
	Error	62	0.21		
21S	Rep	2	0.55	1.71	ns
	Genotype	31	0.32	1.00	
	Error	62	0.32		
21T	Rep	2	0.88	2.75	ns
	Genotype	31	0.33	1.03	
	Error	62	0.32		

[z] Hb, Broad-sense heritability

[y] $p < 0.01$

[x] $p < 0.05$

[w] ns, not significant

MSMA Tolerance in Advanced Breeding Lines Developed in the U.S. Public Breeding Programs. In three tests (21OV, 21HQ, and 21RB), a total of 52 public Upland cotton lines were tested. In Test 21OV (Table 2), 9 of the 13 Upland lines developed by NMSU possessed some level of MSMA tolerance with ratings ranging from 0.83 to 1.33, similar to Pima cotton. In Test 21HQ (Table 3), as compared to the Pima cotton mean, 7 of 14 advanced breeding lines (including three from the University of Arkansas [UA], two from Louisiana State University [LSU], and two from NMSU) had similar crop injuries. In Test 21RB (Table 4), the crop injury ratings ranged from 1.67 to 3.50, and four lines (one from UA, one from LSU, and two from NMSU) exhibited MSMA tolerance with the lowest ratings (1.67-1.83). In total, 20 public Upland cotton lines from three public cotton breeding programs (four from UA, three from LSU, and 13 from NMSU) were identified to be tolerant to MSMA.

MSMA Tolerance in Advanced Breeding Lines Developed in the New Mexico Cotton Breeding Program. In addition to the 13 New Mexico Upland cotton lines with lower crop injury ratings by MSMA, 124 additional breeding lines were divided in four replicated tests and further evaluated for MSMA tolerance (Tables 5 to 8). Although significant genotypic differences were not detected in the ANOVA within each of the tests (Table 1), five, eight, three, and three lines (total: 19) in tests 21P (Table 5), 21R (Table 6), 21S (Table 7), and 21T (Table 8), respectively, had significantly lower crop injury ratings (1.67-2.33) than the check, Acala 1517-08 (with a rating of 3.00-3.17).

The above results indicate that MSMA tolerance often exists in the current advanced breeding lines developed from the public cotton breeding programs in the U.S. It suggests that some of the parental lines used in cross breeding to develop these lines were tolerant to MSMA herbicide.

Table 2. Mean crop injury rating as a measurement for MSMA tolerance in Test 21OV, Las Cruces, NM, June 2021

Cultivar or Line	Developer	Type	Rating
DP 1845 B3XF	Bayer Crop Science	Upland	2.67
DP 1948 B3XF	Bayer Crop Science	Upland	2.17
DP 2038 B3XF	Bayer Crop Science	Upland	1.83
DP 2055 B3XF	Bayer Crop Science	Upland	3.67
DP 340 Pima	Bayer Crop Science	Pima	0.83
DP 347 RF Pima	Bayer Crop Science	Pima	1.33[z]
DP 359 RF Pima	Bayer Crop Science	Pima	0.50
FM 1730GLTP	BASF	Upland	1.83
FM 2334GLT	BASF	Upland	2.00
FM 2498GLT	BASF	Upland	1.33[z]
ST 4993 B3XF	BASF	Upland	2.67
PHY 332 W3FE	Corteva	Upland	1.83
PHY 400 W3FE	Corteva	Upland	2.83
PHY 443 W3FE	Corteva	Upland	2.67
PHY 807 RF Pima	Corteva	Pima	0.83
PHY 881 RF Pima	Corteva	Pima	0.73
Pima 20R022R2P	Corteva	Pima	0.83
NM20	New Mexico State University	Upland	1.33[z]
NM21	New Mexico State University	Upland	2.00
NM22	New Mexico State University	Upland	1.33[z]
NM23	New Mexico State University	Upland	2.17
NM24	New Mexico State University	Upland	1.00[z]
NM25	New Mexico State University	Upland	1.17[z]
NM26	New Mexico State University	Upland	1.00[z]
NM27	New Mexico State University	Upland	1.17[z]
NM28	New Mexico State University	Upland	1.17[z]
NM29	New Mexico State University	Upland	0.83[z]
NM30	New Mexico State University	Upland	3.00
NM31	New Mexico State University	Upland	1.00[z]
NM32	New Mexico State University	Upland	1.83
NMSI 1331	New Mexico State University	Pima	0.83
NMSI 20-01	New Mexico State University	Pima	0.40
	LSD 0.05		0.90
	CV (%)		34.91

[z] **Not significantly different from the mean of Pima cotton at $p > 0.05$**

Consistency of Genotypic Responses to MSMA. The consistency of the results in this study can be gauged by seven commercial cultivars (four Upland: DP 1948 B2XF, FM 1730GLTP, PHY 332 W3FE, and PHY 400 W3FE; and three Pima: DP 347 RF, DP 359 RF, and PHY 881 RF) that were tested in both the 21OV and 21HQ tests (Tables 2 and 3). Similar results, except for FM 1730GLTP, were observed. PHY 400 W3FE also was tested under two different entry identifications in 21HQ with similar results (Table 3). In addition, Acala 1517-08 as a common check across the four AYT (21P, 21R, 21S, and 21T) showed similar results (with ratings between 3.00-3.17). Further, the coefficients of variation (CV) across the seven tests ranged from 17 to 35% with an average of 24% (Tables 2 to 8), similar to cotton yield field trials.

Recovery from Crop Injury by MSMA. For all lines with varied levels of crop injuries after the foliar application of MSMA, no permanent damage was observed. The crop injury symptoms were all transient and disappeared within 3 to 4 wk after herbicide application.

Table 3. Mean crop injury rating as a measurement for MSMA tolerance in Test 21HQ, Las Cruces, NM, June 2021

Cultivar or Line	Developer	Type	Rating
DG 3520 B3XF	Dyna-Gro Seed	Upland	3.17
DP 1646 B2XF	Bayer Crop Science	Upland	1.50[z]
DP 1948 B3XF	Bayer Crop Science	Upland	2.67
DP 2012 B3XF	Bayer Crop Science	Upland	2.17
FM 1730GLTP	BASF	Upland	3.00
FM 1830GLT	BASF	Upland	2.33
NG 4936 B3XF	Americot	Upland	1.83
PHY 332 W3FE	Corteva	Upland	1.33[z]
PHY 390 W3FE	Corteva	Upland	1.17[z]
PHY 400 W3FE-1	Corteva	Upland	2.17
PHY 400 W3FE-2	Corteva	Upland	2.67
PHY 764 WRF	Corteva	Upland	1.50[z]
Pima PHY 881 RF	Corteva	Pima	0.83
Pima DP 341 RF	Bayer Crop Science	Pima	0.83
Pima DP 347 RF	Bayer Crop Science	Pima	0.83
Pima DP 359 RF	Bayer Crop Science	Pima	0.50
ST 45500GLTP	BASF	Upland	1.73
ST 4990B3XF	BASF	Upland	2.67
ARK 1303-29	University of Arkansas	Upland	1.50[z]
ARK 1311-26	University of Arkansas	Upland	1.33[z]
ARK 1319-59	University of Arkansas	Upland	0.93[z]
LA 160063006	Louisiana State University	Upland	1.33[z]
LA 17063008	Louisiana State University	Upland	1.67
LA 17063090	Louisiana State University	Upland	1.17[z]
NM 18B1592	New Mexico State University	Upland	0.90
NM 18B1593	New Mexico State University	Upland	1.17[z]
NM11	New Mexico State University	Upland	1.83
NM12	New Mexico State University	Upland	1.50[z]
NM13	New Mexico State University	Upland	1.67
TAM 12KJ-Q14-2015-708-10	Texas A&M University	Upland	1.67
TAM 14H-11	Texas A&M University	Upland	1.83
TAM KH-14-A-176-2015-314-29	Texas A&M University	Upland	2.00
	LSD 0.05		0.82
	CV (%)		29.95

[z] Not significantly different from the mean of Pima cotton at $p > 0.05$

Table 4. Mean crop injury rating as a measurement for MSMA tolerance in Test 21RB, Las Cruces, NM, June 2021

Cultivar or Line	Developer	Rating
Ark 1301-16	University of Arkansas	2.50
Ark 1311-18	University of Arkansas	2.17
Ark 1308-58	University of Arkansas	1.83
Ark 1317-31	University of Arkansas	1.67[z]
Ark 1309-56	University of Arkansas	2.33
CSX5432	CSIRO, Australia	2.50
TAMLBB16507	Texas A&M University, Lubbock	3.00
TAMLBB17206	Texas A&M University, Lubbock	2.50
OA-11	O & A Enterprises Inc.	3.00
OA-13	O & A Enterprises Inc.	2.67
OA-133	O & A Enterprises Inc.	2.83
GA 2015026	University of Georgia	2.33
GA 2016029	University of Georgia	2.83
GA 2016090	University of Georgia	3.50
TAM 14B-72	Texas A&M University, College Station	2.33
TAM 14E-12	Texas A&M University, College Station	2.00
LA19073002	Louisiana State University	2.83
LA19073070	Louisiana State University	1.67[z]
MS 2010-87-37	Mississippi State University	3.00
MS 2010-87-42	Mississippi State University	2.00
MS 2010-87-5	Mississippi State University	2.17
MS 2010-66-16	Mississippi State University	2.67
MS 2010-28-27	Mississippi State University	2.33
MS 2010-96-9	Mississippi State University	2.83
DP 393	Bayer Crop Science	2.67
DP 493	Bayer Crop Science	2.50
FM 958	BASF	2.33
UA 222 (Check)	University of Arkansas	2.67
NM01	New Mexico State University	2.00
NM02	New Mexico State University	2.00
NM03	New Mexico State University	1.67[z]
NM04	New Mexico State University	1.67[z]
	LSD 0.05	0.94
	CV (%)	24.05

[z] Significantly different from the check, UA 222, at $p < 0.05$

Table 5. Mean crop injury rating as a measurement for MSMA tolerance in Test 21P, Las Cruces, NM, June 2021

Line	Line ID	Developer	Rating
20Y1040-B	AYT01	New Mexico State University	2.50
20Y1091-B1	AYT02	New Mexico State University	2.00[z]
20P1735-B1	AYT03	New Mexico State University	3.00
20Y1095-B	AYT04	New Mexico State University	2.50
20Y1101-B1	AYT05	New Mexico State University	2.83
20Y1101-B2	AYT06	New Mexico State University	2.17[z]
20Y1102-B	AYT07	New Mexico State University	2.33
20Y1103-B1	AYT08	New Mexico State University	3.00
20Y1103-B2	AYT09	New Mexico State University	2.33
20Y1104-B1	AYT10	New Mexico State University	2.17[z]
20Y1105-B	AYT11	New Mexico State University	2.67
20Y1108-B	AYT12	New Mexico State University	2.33
20Y1110-B1	AYT13	New Mexico State University	2.33
20Y1110-B2	AYT14	New Mexico State University	3.00
20Y1219-B1	AYT15	New Mexico State University	2.50
20Y1219-B2	AYT16	New Mexico State University	2.50
20Y1220-B1	AYT17	New Mexico State University	2.50
20Y1220-B2	AYT18	New Mexico State University	2.83
20Y1285-B1	AYT19	New Mexico State University	2.17[z]
20Y1285-B2	AYT20	New Mexico State University	2.33
20Y1128-B1	AYT21	New Mexico State University	3.33
20Y1128-B2	AYT22	New Mexico State University	2.83
20P1752-B	AYT23	New Mexico State University	3.17
20Y1158-B1	AYT24	New Mexico State University	2.33[z]
20T1034-B1	AYT25	New Mexico State University	2.67
20T1050-B1	AYT26	New Mexico State University	2.67
20T1078-B1	AYT27	New Mexico State University	2.67
20T1078-B2	AYT28	New Mexico State University	2.50
20T1108-B1	AYT29	New Mexico State University	2.83
20T1123-B	AYT30	New Mexico State University	3.00
20T1148-B	AYT31	New Mexico State University	2.33
Acala 1517-08	Check	New Mexico State University	3.00
	LSD 0.05		0.75
	CV (%)		17.62

[z] Significantly lower than the check, Acala 1517-08 at $p < 0.05$

Table 6. Mean crop injury rating as a measurement for MSMA tolerance in Test 21R, Las Cruces, NM, June 2021

Line	Line ID	Developer	Rating
20L1101-B	AYT32	New Mexico State University	2.83
20L1001-B1	AYT33	New Mexico State University	2.67
20L1001-B2	AYT34	New Mexico State University	2.50
20L2025-B1	AYT35	New Mexico State University	2.17[z]
20P1752-B	AYT36	New Mexico State University	2.50
20L3007-B1	AYT37	New Mexico State University	2.50
20L3007-B2	AYT38	New Mexico State University	1.83[z]
20L3016-B1	AYT39	New Mexico State University	2.50
20L3016-B2	AYT40	New Mexico State University	2.50
20K1004-B1	AYT41	New Mexico State University	2.50
20K1004-B2	AYT42	New Mexico State University	3.17
20K2001-B1	AYT43	New Mexico State University	3.00
20K2001-B2	AYT44	New Mexico State University	2.67
20P1758-B	AYT45	New Mexico State University	2.67
20K2002-B2	AYT46	New Mexico State University	2.83
20K2030-B1	AYT47	New Mexico State University	2.33[z]
20K2030-B2	AYT48	New Mexico State University	3.17
20K3007-B1	AYT49	New Mexico State University	2.83
20P1520-B	AYT50	New Mexico State University	3.00
20K3030-B1	AYT51	New Mexico State University	2.33[z]
20K3030-B2	AYT52	New Mexico State University	2.00[z]
20P1524-B	AYT53	New Mexico State University	2.83
20K3030-B1	AYT54	New Mexico State University	2.17[z]
20V1013-B	AYT55	New Mexico State University	2.83
20V1019-B1	AYT56	New Mexico State University	2.33[z]
20V1019-B2	AYT57	New Mexico State University	2.50
20V1024-B	AYT58	New Mexico State University	2.67
20V1030-B	AYT59	New Mexico State University	2.33[z]
20V1030-B2	AYT60	New Mexico State University	2.83
20V1031-B1	AYT61	New Mexico State University	2.83
20V1031-B2	AYT62	New Mexico State University	2.83
Acala 1517-08	Check	New Mexico State University	3.17
	LSD0.05		0.76
	CV (%)		17.42

[z] Significantly lower than the check, Acala 1517-08 at $p < 0.05$

Table 7. Mean crop injury rating as a measurement for MSMA tolerance in Test 21S, Las Cruces, NM, June 2021

Line	Line ID	Developer	Rating
20V1168-B1	AYT63	New Mexico State University	2.00[z]
20V1168-B2	AYT64	New Mexico State University	2.50
20V1189-B	AYT65	New Mexico State University	2.50
20P1610-B1	AYT66	New Mexico State University	2.50
20V1191-B	AYT67	New Mexico State University	2.67
20Z1002-B1	AYT68	New Mexico State University	2.50
20Z1002-B2	AYT69	New Mexico State University	2.33
20P1622-B1	AYT70	New Mexico State University	2.83
20Z1057-B2	AYT71	New Mexico State University	2.00[z]
20P1622-B2	AYT72	New Mexico State University	3.00
20P1634-B	AYT73	New Mexico State University	2.67
20Z1066-B1	AYT74	New Mexico State University	2.83
20Z1066-B2	AYT75	New Mexico State University	2.67
20Z1067-B1	AYT76	New Mexico State University	2.33
20Z1067-B2	AYT77	New Mexico State University	2.83
20Z1067-B3	AYT78	New Mexico State University	2.50
20Z1068-B1	AYT79	New Mexico State University	2.17
20Z1068-B2	AYT80	New Mexico State University	3.17
20Z1074-B	AYT81	New Mexico State University	2.33
20P1700-B1	AYT82	New Mexico State University	2.67
20Z1076-B1	AYT83	New Mexico State University	2.33
20Z1076-B2	AYT84	New Mexico State University	2.50
20Z1088-B1	AYT85	New Mexico State University	2.17
20Z1088-B2	AYT86	New Mexico State University	2.00[z]
20Z1089-B1	AYT87	New Mexico State University	2.17
20Z1089-B2	AYT88	New Mexico State University	2.67
20Z1095-B1	AYT89	New Mexico State University	3.00
20Z1095-B2	AYT90	New Mexico State University	3.00
20Z1139-B1	AYT91	New Mexico State University	3.00
20Z1139-B2	AYT92	New Mexico State University	2.67
20Z1169-B1	AYT93	New Mexico State University	2.33
Acala 1517-08	Check	New Mexico State University	3.00
	LSD 0.05		0.92
	CV (%)		21.89

[z] Significantly lower than the check, Acala 1517-08 at $p < 0.05$

Table 8. Mean crop injury rating as a measurement for MSMA tolerance in Test 21T, Las Cruces, NM, June 2021

Line	Line ID	Developer	Rating
20V1168-B1	AYT63	New Mexico State University	2.00[z]
20V1168-B2	AYT64	New Mexico State University	2.50
20V1189-B	AYT65	New Mexico State University	2.50
20P1610-B1	AYT66	New Mexico State University	2.50
20V1191-B	AYT67	New Mexico State University	2.67
20Z1002-B1	AYT68	New Mexico State University	2.50
20Z1002-B2	AYT69	New Mexico State University	2.33
20P1622-B1	AYT70	New Mexico State University	2.83
20Z1057-B2	AYT71	New Mexico State University	2.00[z]
20P1622-B2	AYT72	New Mexico State University	3.00
20P1634-B	AYT73	New Mexico State University	2.67
20Z1066-B1	AYT74	New Mexico State University	2.83
20Z1066-B2	AYT75	New Mexico State University	2.67
20Z1067-B1	AYT76	New Mexico State University	2.33
20Z1067-B2	AYT77	New Mexico State University	2.83
20Z1067-B3	AYT78	New Mexico State University	2.50
20Z1068-B1	AYT79	New Mexico State University	2.17
20Z1068-B2	AYT80	New Mexico State University	3.17
20Z1074-B	AYT81	New Mexico State University	2.33
20P1700-B1	AYT82	New Mexico State University	2.67
20Z1076-B1	AYT83	New Mexico State University	2.33
20Z1076-B2	AYT84	New Mexico State University	2.50
20Z1088-B1	AYT85	New Mexico State University	2.17
20Z1088-B2	AYT86	New Mexico State University	2.00[z]
20Z1089-B1	AYT87	New Mexico State University	2.17
20Z1089-B2	AYT88	New Mexico State University	2.67
20Z1095-B1	AYT89	New Mexico State University	3.00
20Z1095-B2	AYT90	New Mexico State University	3.00
20Z1139-B1	AYT91	New Mexico State University	3.00
20Z1139-B2	AYT92	New Mexico State University	2.67
20Z1169-B1	AYT93	New Mexico State University	2.33
Acala 1517-08	Check	New Mexico State University	3.00
	LSD 0.05		0.92
	CV (%)		22.15

[z] Significantly lower than the check, Acala 1517-08 at $p < 0.05$

DISCUSSION

MSMA has been used in cotton production since the 1960s; it is known to cause transient crop injury including stunting, leaf purpling, and stem reddening. Seedlings typically recover within 4 to 5 wk of MSMA treatment. However, long-term adverse effects due to MSMA have been observed consistently, including reduction in cotton yield, plant height, boll weight, boll retention, and delays in maturity, especially when MSMA is applied over the top repeatedly after the early seedling stage or in high concentrations. Therefore, identification of MSMA-tolerant cotton cultivars and lines will allow selection of commercial cultivars for production where MSMA is used. Further identification of elite breeding lines with MSMA tolerance is a prerequisite for understanding the genetic basis governing MSMA tolerance.

Because the present study was the first in cotton concerning genetic differences in MSMA tolerance, it is currently unknown if any morphological, physiological, and biochemical characteristics are related to the tolerance. Based on an early study on uptake and translocation of [14C] MSMA in cotton, Keese and Camper (1994) first suggested that the cotton leaf cuticle could play a part in the MSMA tolerance mechanism. However, Keese and Camper (2006) later refuted this conclusion, as minimal change in cuticle concentrations was observed. Camper et al. (2004) then suggested that cotton tolerance to MSMA could be related to glutathione synthetase activity and possibly to the presence of phytochelatins. However, Pima cotton leaves usually contain more waxy cuticles on the surface, which could reduce absorption of MSMA in leaves and therefore prevent crop injury. This might not explain why some Upland cotton cultivars and lines were tolerant, because differences in cuticle contents within Upland cotton are low. Nimbal et al. (1995) showed that organic MSMA-resistant and -susceptible Mississippi biotypes of common cocklebur did not differ in uptake, translocation, and metabolic degradation, which were therefore not involved in the mechanism of resistance of the MSMA-resistant biotype. There might be other morphological and physiological traits on the leaf surface that are associated with MSMA tolerance in cotton. In this study, a few Upland cotton lines were nectariless or glandless, and lines also differed in trichome density on leaves. These morphological traits appeared to be unrelated to responses to MSMA. Further studies are needed to investigate the genetic, physiological, and molecular basis of MSMA tolerance in cotton.

Because the effectiveness of MSMA on weed control is dependent on environmental factors such as temperature and crop growth stage, reliability of results in MSMA tolerance of cotton was addressed in this study. First, a total of seven tests with more than 200 cultivars and lines (in contrast to the few genotypes used in many previous studies on weed control in cotton production) were evaluated. Second, each test was replicated three times, and blocking effects due to replications were detected in four tests, suggesting that blocking control was effective in estimating systematic experimental errors using the randomized complete block design. Third, significant genotypic variation was detected in three tests, indicating consistent responses of the same set of genotypes within each of the three tests. To further address the consistency of our results, eight cultivars were included in more than one test, and consistent results were observed between/among tests for each genotype except for one cultivar. The finding that all Pima cotton tested displayed tolerance but Upland cotton is sensitive, is new and should be useful to cotton production and breeding. In addition, several Upland cotton lines and cultivars were tolerant, indicating genetic variation within the Upland cotton species. The authors speculate that the MSMA tolerance in Pima cotton and several Upland cotton cultivars detected in this study can be qualitative in nature and controlled by major gene(s) and environmental effect. The genotype-by-environment interaction can be lower than quantitative tolerance for a typical agronomic trait such as lint yield. An indirect piece of evidence is that a major tolerance gene for Envoke® (trifloxysulfuron-sodium, Syngenta Crop Protection, Greensboro, NC) in cotton was identified and cloned by Thyssen et al. (2014, 2018). Therefore, results in this study provide a set of tolerant germplasm lines to study the genetic and genomic basis of MSMA tolerance in cotton.

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DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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