Genetic Improvement of New Mexico Acala Cotton Germplasm and Their Genetic Diversity

J. F. Zhang, Y. Lu, H. Adragna, and E. Hughes

ABSTRACT

The New Mexico cotton breeding program was established in 1926 and has been led by five generations of breeders and geneticists. The program has released more than 30 Acala 1517 cotton (Gossypium hirsutum L.) cultivars and numerous germplasm lines known for high fiber quality and Verticillium wilt (caused by Verticillium dahliae Kleb.) tolerance that have made substantial contributions to cotton breeding in the USA. The present project was initiated in 2003 to evaluate the genetic improvement of Acala 1517 cultivars and lines released over the past 75 yr in yield, boll size, seed index, lint percentage, fiber length, fiber strength, and micronaire. Their genetic divergence was also estimated by simple sequence repeat (SSR) markers. On the basis of the data available from annual yield trials, lint yield and lint percentage in Acala 1517 cotton have steadily increased since the 1930s, while boll size and seed index have gradually decreased since the 1960s. Fiber strength has been enhanced since the 1960s, which has been accompanied by steady increase in micronaire. However, fiber length in Acala 1517 cultivars tended to shorten from 31.0 to 30.0 mm from 1960 to 1990, whereas newly released Acala 1517 cultivars (Culp et al., 1996; Meredith, 2000) further showed that the overall yield increases from 1945 to 1978 (Culp and Green, 1992). For California Acala cotton, Bassett and Hyer (1985) estimated the genetic gain of 8.0 kg ha$^{-1}$ yr$^{-1}$ from 1930 to 1980. In the Mississippi Delta, genetic gain in lint yield improvements averaged 9.1 to 10.2 kg ha$^{-1}$ yr$^{-1}$ from 1922 to 1966 and 8.5 to 9.5 kg ha$^{-1}$ yr$^{-1}$ from 1910 to 1978; however, the rate was shown to be decreased when a longer period was included in the analysis: 5.4 kg ha$^{-1}$ yr$^{-1}$ from 1938 to 1993 and 4.7 kg ha$^{-1}$ yr$^{-1}$ from 1938 to 1999. The genetic gain was further decreased to 3.5 kg ha$^{-1}$ yr$^{-1}$ from 1983 to 1999 (Bridge et al., 1971; Bridge and Meredith, 1983; Meredith 2000). The slowed genetic gain in cotton yield improvement was thought to be due to narrow genetic base of upland cotton and repeated use of a few upland cotton germplasm in major commercial cotton breeding programs (May et al., 1995; Meredith, 2000; Lewis, 2001).

The success of cotton breeding for high yield before the 1990s was in part attributed to free exchanges of germplasm and broad use of parental lines. For example, Acala cotton germplasm released from the New Mexico State University cotton breeding and genetics program has played an important role in the U.S. cotton industry. Since the establishment of the program in 1926, more than 30 Acala 1517 series of cotton cultivars and numerous germplasm lines have been developed and released (Staten, 1970; Smith et al., 1999). New Mexico Acala cotton germplasm, known for its high fiber quality, good Verticillium wilt tolerance, and large boll size (Smith et al., 1999), is adapted to the southwestern growing region (semiarid and hot in the summer) of the U.S. Cotton Belt. Even though they are very tall and late-maturing with low yield when grown in other regions, they have been used extensively as the parental lines for developing other types of cotton cultivars since the 1950s. On the basis of Bowman et al. (1996), approximately 45% of cotton cultivars including most California Acala cotton released in the USA from 1950 to 1990 contained New Mexico cotton germplasm in their pedigrees. However, no information is available on the genetic gain in lint yield and other traits of agronomic importance in Acala cotton cultivars and germplasm lines released in the New Mexico Cotton program.

The uniqueness of the Acala cotton is mostly due to its unique breeding history in which germplasm from G. barbadense L. and Triple Hybrid (ATH, G. arboreum)

Abbreviations: JC; Jaccard coefficient; SSR, simple sequence repeats.
L. × *G. thurberi* Todaro × *G. hirsutum* L.) were introgressed into the Acala cotton (Smith et al., 1999). Interspecific introgression was also evident in the development of high quality Pee Dee germplasm lines (Culp and Green, 1992; May, 2001). There have been attempts to introduce fiber quality genes from Acala and/or Pee Dee lines into other cottons to develop high-yielding cultivars, but success has been limited (Bowman and Gutierrez, 2003). Priority in Acala cotton breeding programs has been given to developing germplasm with better fiber quality in which desirable genes for fiber quality have been maintained and accumulated. Zhang et al. (2005) reported that some representative commercial Acala cotton cultivars had a high frequency of several unique SSR markers that were associated with fiber quality traits. However, information on genetic divergence among the historically released Acala cultivars and germplasm is lacking. The assessment of genetic diversity in commercial Acala cotton and germplasm released during various periods on the basis of molecular markers will provide useful information for sustainable future cotton breeding and germplasm conservation. Detailed DNA marker information could provide clues in identifying certain chromosomal regions in Acala cottons that might be associated with their agronomic performance.

The objectives of this study were (i) to analyze the progress in genetic improvement of Acala 1517 cotton cultivars released from New Mexico since 1930 and (ii) to assess their genetic divergence on the basis of SSR DNA markers.

**MATERIALS AND METHODS**

**Replicated Field Trials**

Regional tests on Acala 1517 experimental strains and cultivars were conducted each year since the mid-1930s on the University farm near Las Cruces, NM, and on the Artesia Agricultural Science Center, Artesia, NM. The tests at different locations and in different years had different cultivars and lines. The cultivars were arranged in a randomized complete block design with four replicates. The individual plot size was two or four rows, each 10 to 15 m long. When cotton was mature, 50 open bolls were hand harvested from each of the plots for measuring lint percentage and fiber quality. Fiber quality traits including length, strength, and micronaire (fineness) were tested with in-house single instruments (fibrograph, micronaire, and stelometer). In earlier years, the two center rows were hand-picked for yield determination. Since the mid-1970s, each of the plots was mechanically harvested for seed-cotton yield. In earlier years, the two center rows were hand-picked for yield determination. Since the mid-1970s, each of the plots was mechanically harvested for seed-cotton yield estimates. The data were subjected to an analysis of variance (ANOVA) manually or by SAS (SAS, Cary, NC) or AgroBase 21 (Agronomix Software Inc., Winnipeg, MB).

During different testing periods, different standard cultivars were used. From the late 1930s to 1950s, Acala 1517 was the most used standard for comparison, while Acala 1517V was the standard cultivar from 1965 to 1974. From the late 1970s to 1980s, Acala 1517-75 and 1517-77 were commonly used. Acala 1517-91, 1517-95, and 1517-99 have been frequently used as standards since the 1990s. For comparison purposes, the yield performance of each cultivar was adjusted as percentages in relation to the yield of the standard used in the same tests. Acala 1517V served as the common standard for overall adjustments for yield and other traits. First, the percentage of yield from the selected standards (e.g., CK1) over 1517V in different periods was calculated (p1 = CK1/1517V). Then, percentage (p2) of yield from tested cultivars (e.g., 1517) over the selected standards (e.g., CK1) was calculated (p2 = 1517/CK1). Finally, the adjusted yield in comparison with 1517V for a tested cultivar equaled to the unadjusted yield 1517 × p1 × p2. Information was also collected from data published in *Crop Science* for cultivar registrations (Table 1).

**SSR Fingerprinting**

Seeds for 31 cotton germplasm lines including the 16 Acala cultivars listed in Table 1 and 13 other Acala cultivars or strains (Acala Original, Acala Mesilla Valley, Acala 2, Acala 8, Acala 44WR, Acala 51, Acala 1517-5-12, Acala 1481, Acala Hopi 76-18-1, Acala SJ-2, Acala SJ-3, Acala SJ-4, and Acala Maxxa) were provided by Dr. E. Percival for SSR fingerprinting (USDA-ARS, Southern Crops Research Laboratory, Crop Germplasm Research Unit, College Station, TX). TM-1, the genetic standard of upland cotton (Kohel et al., 1970), and NM 24016, an interspecific-derived Acala cotton genetic stock (Cantrell and Davis, 2000), were also included for comparison purposes.

The cotton genotypes tested were grown in the greenhouse in 2003 and leaf tissues from at least 10 plants per line were harvested. Genomic DNA was extracted from the bulked leaves by the micro-prep method as described by Zhang and Stewart (2000). The DNA concentration was determined by a fluorometer.

Sixty-three pairs of BNL SSR primers, labeled with fluorescent HEX (4,7,2’4’5’7’-hexafluoro-6-carboxyfluorescein), NED (7’8’-benzo-5’ fluoro-2’4’7-trichloro-5-carboxyfluorescein), or TAM (6-carboxyfluorescein), were selected for the present study. On the basis of Liu et al. (2000b), these SSR primers were chosen to amplify fragments that were distributed on most of the known chromosomes with 2 to 4 markers per chromosome. This ensured broad genome coverage of genotyping for representative estimation of genetic distance. The PCR reactions were performed with a thermal cycler (PerkinElmer 9600 Thermocycler; PerkinElmer, Foster City, CA) in a reaction solution containing 80 ng DNA template, 0.15 mM primers, 0.2 mM each dNTPs, 1× GeneAmp PCR Buffer, 2.5 mM MgCl2, and 0.5 units of AmpliTaq DNA polymerase (PerkinElmer). The PCR conditions were as follows: 7 min at 95°C, followed by 40 cycles of 15 s at 94°C for DNA denaturing, 30 s at 55°C for primer annealing, and 2 min at 72°C for extension with a final extension for 30 min at 72°C. The finished PCR samples were stored at −20°C until use.

The PCR products were separated by polyacrylamide gel electrophoresis with an ABI377 Sequencer (PerkinElmer). For technical details see Liu et al. (2000a). Most SSR primers usually amplified one or two major bands, while some gave more than three bands. For the SSR markers, all the alleles were treated independently as a binary variable with 1 for presence and 0 for absence, because heterozygous status for codominant markers in the true breeding cultivars or lines was very rare, if any. Genetic similarity coefficients were calculated on the basis of simple match coefficients (SM) and Jaccard’s coefficient (JC) using the Numerical Taxonomy Multivariate Analysis System (NTSYSpc) Version 2.1 software package (Exeter Software, Setauket, NY). The resulting similarity coefficients were used to perform the cluster analysis by the unweighted pair group method of arithmetic means (UPGMA).
RESULTS AND ANALYSIS

Yield and Yield Components

Yield

Overall, cotton yield in Acala 1517 cultivars has increased at an average rate of 1.4% per year from 1930 to 2004 (Table 2). However, from Fig. 1, it is apparent that two periods of yield improvement existed. In the first period from 1930 to 1982, the increment rate for yield was 0.77% per year, whereas in the second period from 1982 to present, the gain in yield advancement increased to 3.1% per year. However, the differences in yield of cultivars across years cannot only be attributed to the genetic gain. Agronomic practices such as fertilizers have been changed over time, which accounted for some of the yield differences.

During the 1970s to 1980s, New Mexico State University had an array of excellent cotton breeders and specialists (N.R. Malm, D.D. Davis, C.E. Barnes, and C.L. Roberts). Their long-lasting and joint efforts eventually paid off and produced many Acala 1517 cultivars (Table 1). As a result, the improvement in cotton yield was accelerated in 1980s and this trend has been maintained through the 1990s when the program was led by R.G. Cantrell.

Boll Size

Boll size was measured on the basis of seedcotton weight (g) per boll or lint weight (g) per boll in the program. However, only 11 cultivars measured by seedcotton weight per boll were used for the analysis (Fig. 2). It appears that boll size has decreased from large boll (>7 g/boll) to medium-sized bolls (5.3-5.5 g/boll) in 2004. The increment rate for boll size is −0.05 g per boll per year (Table 2).

Seed Index

The earliest Acala cotton cultivars had relatively small seed (Fig. 3). However, on the basis of the data available from the late 1960 to the 1990s, the cultivars released from 1970 to the early 1980s had large seed (>13 g of seed index), whereas the seed size in the cultivars released from the late 1980s to the 1990s had smaller seed (about 11.5 g for seed index). The trend with a reduction of 0.10 g per year was linear and highly significant from 1969 to 1999 (Table 2).

Table 1. Acala 1517 cotton cultivars released.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Year released</th>
<th>Pedigree</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acala Young†</td>
<td>1929</td>
<td>Watson’s</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>College Acala</td>
<td>1930</td>
<td>Acala P12</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1064†</td>
<td>1937</td>
<td>Acala Young</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517†</td>
<td>1939</td>
<td>Acala 1064</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517A</td>
<td>1941</td>
<td>Acala 1064</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517WR</td>
<td>1946</td>
<td>Acala 1517</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517B</td>
<td>1949</td>
<td>Watson’s Acala</td>
<td>Staten, 1970; Davis et al., 1978c</td>
</tr>
<tr>
<td>Acala 1517C (7133)</td>
<td>1951</td>
<td>NM 1544 × NM 1577</td>
<td>Staten, 1970; Davis et al., 1978c</td>
</tr>
<tr>
<td>Acala 1517C (8893)</td>
<td>1954</td>
<td>Reselection from 7133</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517BR</td>
<td>1954</td>
<td>ST 20/Acala/Acala 1517WR/Acala 1517B</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517−BR1</td>
<td>1957</td>
<td>Acala 1517BR/Acala 1517C</td>
<td>Staten, 1970; Davis et al., 1978c</td>
</tr>
<tr>
<td>Acala 1517−C (1028)†</td>
<td>1958</td>
<td>Reselection from 7133</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517D†</td>
<td>1960</td>
<td>A cross of two strains of unknown parentage</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517−BR2 (B479)</td>
<td>1961</td>
<td>(8373/ST 20)/Acala 216(Acala 49/Hartsville)</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517V (6612)</td>
<td>1964</td>
<td>Acala 2503/Coquette</td>
<td>Staten, 1970; Malm et al., 1978a</td>
</tr>
<tr>
<td>Acala 1517−BR2 (60-209B)†</td>
<td>1965</td>
<td>Reselection from Acala 1517−BR2</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Hopicala</td>
<td>1965</td>
<td>Acala 1517 selection 5-12/HA76</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517−80</td>
<td>1968</td>
<td>9136/49W</td>
<td>Staten, 1970</td>
</tr>
<tr>
<td>Acala 1517−70†</td>
<td>1969</td>
<td>Acala 2503/Coquette</td>
<td>Staten, 1970; Davis et al., 1978b</td>
</tr>
<tr>
<td>Acala 1517−75†</td>
<td>1970</td>
<td>B1413/Hopicala</td>
<td>Malm et al., 1978b</td>
</tr>
<tr>
<td>Acala 1517−77</td>
<td>1975</td>
<td>Acala 688/Acala 9608</td>
<td>Barnes et al., 1980</td>
</tr>
<tr>
<td>Acala 1517−77BR†</td>
<td>1977</td>
<td>Reselection from Acala 1517−77</td>
<td>Roberts et al., 1982</td>
</tr>
<tr>
<td>Acala 1517−E1</td>
<td>1976</td>
<td>Acala 3080/PD 2165</td>
<td>Davis et al., 1978c</td>
</tr>
<tr>
<td>Acala 1517−E2†</td>
<td>1978</td>
<td>Selection from Acala 1517−E1</td>
<td>Davis et al., 1980</td>
</tr>
<tr>
<td>Acala 1517−SR1</td>
<td>1982</td>
<td>Acala 1517−E1/Unknown storm-proof</td>
<td>Malm et al., 1984</td>
</tr>
<tr>
<td>Acala 1517−SR2</td>
<td>1986</td>
<td>Acala 1517−E1/Unknown storm-proof</td>
<td>Malm et al., 1987</td>
</tr>
<tr>
<td>Acala 1517−SR3†</td>
<td>1990</td>
<td>Acala 1517−E1/Unknown storm-proof</td>
<td>Cantrell et al., 1992</td>
</tr>
<tr>
<td>Acala 1517−88†</td>
<td>1991</td>
<td>Acala 1517−77BR/DP 70</td>
<td>Roberts et al., 1988</td>
</tr>
<tr>
<td>Acala 1517−91†</td>
<td>1991</td>
<td>Acala 8130/Acala 8874</td>
<td>Cantrell et al., 1992</td>
</tr>
<tr>
<td>Acala 1517−95†</td>
<td>1995</td>
<td>From 1517−E2 (3080/PD 2165)</td>
<td>Cantrell et al., 1995</td>
</tr>
<tr>
<td>Acala 1517−99†</td>
<td>1999</td>
<td>B742/E1141</td>
<td>Cantrell et al., 2000</td>
</tr>
<tr>
<td>Acala 1517−02</td>
<td>2004</td>
<td>Prema/(Acala 1517−95/GC-362)</td>
<td>Zhang et al., unpublished data</td>
</tr>
<tr>
<td>Acala 1517−03</td>
<td>2004</td>
<td>B4222/H11014</td>
<td>Zhang et al., unpublished data</td>
</tr>
<tr>
<td>Acala 1517−04</td>
<td>2004</td>
<td>Acala 1517−95/87D3-24</td>
<td>Zhang et al., unpublished data</td>
</tr>
</tbody>
</table>

† Used for SSR fingerprinting.

Table 2. Linear regression between year released and trait performance in Acala 1517 cultivars.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Period</th>
<th>Slope</th>
<th>Correlation</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (% 1517-V)</td>
<td>1930–2004</td>
<td>1.4390</td>
<td>0.9261**</td>
<td>21</td>
</tr>
<tr>
<td>Boll size (g)</td>
<td>1958–2004</td>
<td>−0.0462</td>
<td>−0.9615**</td>
<td>11</td>
</tr>
<tr>
<td>Seed index (g)</td>
<td>1969–1991</td>
<td>−0.0999</td>
<td>−0.8508**</td>
<td>9</td>
</tr>
<tr>
<td>Micronaire (units)</td>
<td>1961–2004</td>
<td>0.0370</td>
<td>0.3132</td>
<td>26</td>
</tr>
<tr>
<td>Fiber length (inch)</td>
<td>1961–2004</td>
<td>0.1202</td>
<td>0.8354**</td>
<td>23</td>
</tr>
<tr>
<td>Fiber strength (g/tex)</td>
<td>1961–2004</td>
<td>0.0001</td>
<td>0.0999</td>
<td>19</td>
</tr>
</tbody>
</table>

* P < 0.05. ** P < 0.01.
**Lint Percentage**

The earliest Acala cultivars had relatively high lint percentage (>40%). However, lint percentage in the cultivars released in the 1940s was reduced to 35 to 36%. Since then, lint percentage in Acala 1517 cultivars has been steadily improved with a rate of 0.12% per year (Table 2, $P < 0.01$) and reached approximately 40% in the cultivars released in the 1990s (Fig. 4). The most recently released cultivars have even higher lint percentage (41–44%).

**Fiber Quality**

**Fiber Length**

Most Acala 1517 cultivars released in the 1960s to 1970s had fiber length greater than 30.99 mm, while the fiber length in the cultivars released in the 1980s was reduced to less than 30.48 mm (Fig. 5). The increment rate was −0.0254 mm per year (Table 2). However, the negative trend was reversed in the newly released cultivars since 1991 that have had longer fiber (>30.73 mm).

**Fiber Strength**

Except for the cultivars released in the late 1980s to early 1990s that had slightly reduced fiber strength, Acala 1517 cultivars had improved fiber strength over time with an increment rate of 0.0294 kN m kg$^{-1}$ yr$^{-1}$ (Table 2; Fig. 6). The most recently released cultivars (1517-02, 03, and 04) had strength between 225.4 to 245.0 kN m kg$^{-1}$.

**Micronaire**

Micronaire is a measurement for fiber fineness and maturity, equivalent to fiber weight per unit fiber length, i.e., the higher the micronaire, the coarser the fiber. Micronaire in Acala 1517 cultivars has been steadily increased over years from 3.8 in 1960 to 4.6 in 2004. The increment rate, 0.01 per year, is linear and highly significant (Table 2; Fig. 7). However, micronaire in the newly released cultivars (1517-02, 03, and 04) is still well below 4.9, a discount point for coarser fiber.

In summary, yield in Acala 1517 cultivars has been steadily increased with a higher genetic gain seen since 1970s. The yield improvement has been accompanied by a steady increase in lint percentage and micronaire, and a reduction in boll and seed size. The new higher yielding cultivars have higher lint percentage, coarser fiber, smaller bolls and smaller seed. Fiber length and strength in the cultivars released since the late 1990 were significantly improved.

**SSR Marker Diversity and Cluster Analysis**

Sixty-three SSR primer pairs generated 86 loci and 189 alleles, among which 68 SSR loci with 154 alleles
are distributed on 45 linkage groups with 2 to 4 loci per chromosome (Liu et al., 2000b; Lacape et al., 2003). The SSR markers were relatively evenly distributed on the A and D subgenomes. Of 37 (84 alleles) and 31 loci (74 alleles) that were distributed on the two subgenomes, respectively, 12 and 11 loci showed polymorphism. Of the 18 loci with 31 alleles that were not assigned to any chromosomes, 9 loci were polymorphic. A total of 32 polymorphic SSR markers were produced in the set of germplasm. On average, each chromosome carried 3.3 SSR markers. Genetic distance between Acala germplasm ranged from 0.065 to 0.380 with an average of 0.193, indicating substantial genetic diversity within Acala cotton germplasm. As evidenced from the pedigree analysis, the high genetic divergence within Acala cotton was in part attributed to interspecific germplasm introgression into the Acala cotton.

Direct selection played a very important role in developing Acala cultivars, especially in the early days. Of 30 Acala 1517 cultivars, 13 were from direct pedigree selections. Except for 1517BR and its derivative, 1517-BR1, all the early Acala cultivars (before 1960) including College Acala, Acala 1064, Acala 1517, Acala 1517A, Acala 1517WR, and Acala 1517B were from reselections. Their original progenitor can be traced back to Watson. However, Acala Original and Acala 1064 were grouped with TM-1, an inbred line from many generations of self-pollination from DP 14. Acala Young, a reselection from Watson, was grouped with Acala SJ-3, while Acala 1517 WR was grouped with Acala SJ-2. This indicated that the original Acala population contained enormous genetic variation and within-population reselection dramatically changed genotypic composition, resulting in great dissimilarity between original heterozygous and heterogeneous population and its selections.

Acala Young formed a large group with 16 other Acala germplasm, denoted as New Mexico Acala group. This group can be further divided into six subgroups on the basis of the genetic similarities (Fig. 8).

**Subgroup (1)**

Acala 2, 1517-5-12, 1517-BR2, and 1517-E2. This subgroup (similarity ranging from 0.784-0.835), except for Acala 2 that was also a selection from the original Acala introduction, can trace all the pedigree to Acala 1517. In addition, 1517-BR2 and 1517-E2 had *G. barbadense* (Pima or/and Tanguis) germplasm introgression.

**Subgroup (2)**

Acala 1517(NM), 1517D, 1517-75, 1517C, 1517-91, and 1517-SR3. In this group, 1517C was a breeding line
selected from a cross between two selections from 1517 and it produced 1517D with *G. barbadense* introgression (JC between C and D = 0.883). *Gossypium barbadense* germplasm introgression into 1517 selection also gave rise to 1517V, the first Verticillium wilt tolerant cultivar. The same 1517 selection resulted in Acala 1517-75 when crossed with DP 14 germplasm. Acala 1517V gave rise to 8874 that in turn produced 1517-91 when crossed with a selection from 1517-70. Selection from Acala 1517D also produced Acala SJ-1 and SJ-2. 1517V, 1517-75, and 1517-91 were related through breeding line 2503, which was a selection from 1517. Acala 1517C was more similar to Acala 1517-75 and 1517-91 (JC = 0.835 and 0.918, respectively) than Acala 1517D was (JC = 0.802 and 0.847, respectively). 1517-SR3 had 1517-E1 background which was derived from a cross involving 3080.

**Subgroup (3)**

Acala 1517-70, 1517-77BR, and 1517-88. 1517-70 had Hopicala background and 1517-77 was its direct selection which in turn gave 1517-77BR via direct selection. Acala 1517-88 was developed from a cross of 1517-77BR and DP 70. However, 1517-88 and 1517-77BR were highly similar (JC = 0.931), while 1517-77BR was less similar to 1517-70 (JC = 0.835).

**Subgroup (4)**

Acala 1481.

**Subgroup (5)**

Acala 1517-99. It has Acala 9136 in the pedigree which gave 3080. 9136 had significant *G. barbadense* germplasm introgression. 1517-E2 also had 3080 background, but it was not close to Acala 1517-99, although they fell into the same large NM Acala group.

**Subgroup (6)**

Acala SJ-3 and Acala Young. Acala SJ-3 had 1517V in its pedigree, and 1517V can be traced back to Acala 1517 which was a selection from Acala Young.

The second group involved cultivars from both New Mexico and California, including Acala 51, Acala 4-42, Acala SJ-2, Acala 1517WR, Acala Mesilla Valley, and Acala Maxxa (Fig. 8). This is referred to as NM/CA group. Acala 51 was derived from a cross between a
selection from Acala 8 and a Delta upland cotton type (Missdel). Acala 8 was a direction selection from the original Acala introduction (1908) which gave rise to Acala 9 which in turn produced Acala 1517. However, Acala 51 was not grouped together with Acala 8. SJ-2 and Maxxa were related to Acala 51 through I-2302 in its pedigree. Acala 4-2 was a direct selection from Acala 1517 in California. Acala 1517-5-17 and Acala 1517WR were re-selections from 1517. Acala Mesilla Valley was a selection from selection derived from Watson.

The third group included Acala 44WR and 1517-95 (Fig. 8). Zhang et al. (2005) reported that the four most recently released New Mexico Acala cultivars (1517-95, 1517-99, 1517-02, and 1517-03) were as dissimilar to one another as to commercial cultivars from other sources. Compared with other commercial cultivars, Acala 1517-99 was more similar to Acala 1517-95 since both had a common ancestor germplasm (Acala 9130) in their pedigrees. Both were also relatively similar to 1517-03, but these three were highly dissimilar to 1517-02, even though 1517-02 had 1517-95 in its pedigree. In the present study where more than 30 Acala germplasm lines were genotyped, 1517-95 was not grouped together with 1517-99, although they were grouped together with most of the other Acala germplasm to form a giant Acala family.

The fourth group included Acala Original, TM-1, and Acala 1064 (Fig. 8). Acala 1064 was a selection from Young, but it was not grouped with its descendents. Acala 1064 was most distant from other Acala germplasm, but relatively closer to TM-1 and Acala Original (JC = 0.77–
This could indicate that the earlier Acalas were more similar to DP types before germplasm from Pima, Tanguis and Triple Hybrid was introduced into Acalas.

Other germplasm, Acala 8, Acala SJ-4, Acala Hopi 76-18-1, and NM 24016, each formed separate groups (Fig. 8). SJ-4 had the Triple Hybrid background. Hopiaca had Hopi and Acala 1517 backgrounds. Acala 8 was a direct selection from Acala (1906) and it was also contained in the pedigree of SJ-4. However, SJ-4 contained complex germplasm sources including Pima, Tanguis, and Triple Hybrid backgrounds. Therefore, this California Acala cultivar was distant from others and formed a separate group. Acala 2, 8, and 51 were generally distant from other Acala genotypes, but closer to other California and earlier Acala germplasm. Acala Hopi was the most dissimilar to others with JC ranging from 0.62 to 0.77, indicating that this germplasm is the most diverse germplasm in Acala cotton.

DISCUSSION

Comparing the performance of obsolete and current cultivars and analyzing annual variety trials not only provide detailed information on genetic gain in yield and fiber quality improvement, but also shed light into trends of trait changes over time. This should enable breeders and geneticists to evaluate breeding progress that has been achieved, and review and design their breeding strategies in terms of parental line selection, population development, and selection methods. Annual variety trials usually have more than 2 yr data across multiple locations, which provide reliable estimates on performance of newly released cultivars and breeding lines, and also can accommodate many more lines to be compared. However, the drawbacks are that (i) at least two common standard cultivars should be used during consecutive testing years and (ii) genotype × environment interaction could have different effects on performance of different cultivars. Furthermore, this analysis assumes that tested cultivars and standards have similar linear responses to environments, so that yield and other traits for tested cultivars can be linearly adjusted on the basis of the common standards. Thus, historical yield data over years have these limitations and do not necessarily represent genetic gain over time when regression analysis is conducted. The most appropriate way of assessing the true yield potential in all the genotypes is to evaluate them in the same environments across years and locations. Further field tests will be conducted to estimate more accurately the genetic gains sustained in the Acala cotton germplasm. However, since the most current production practices will be followed, to which new cultivars are more adapted, the yield potential for obsolete cultivars could be underestimated.

After comparing obsolete and modern cotton cultivars grown in the Mississippi Delta, Bridge et al. (1971) and Bridge and Meredith (1983) indicated that high-yielding modern cultivars had higher lint percentage, smaller bolls and seed, and higher micronaire values. Yield improvement over time was mainly due to the increase in lint percentage, number of bolls per plant, and early maturity (Bridge et al., 1971; Hoskinson and Stewart, 1977; Wells and Meredith, 1984c; Culp and Green, 1992). Fiber length and strength showed little change over time, except that fiber strength in the Pee Dee germplasm was enhanced (Culp and Green, 1992). In the present study, on the basis of the data available from annual yield trials in New Mexico, our analysis shows that lint yield and lint percentage in Acala 1517 cotton have been steadily increased at an annual rate of 1.4 and 0.04% between 1930 and 2004, respectively, while boll size and seed index have been gradually reduced since the 1960s. Yield improvement can be divided into two periods, 1930 to 1982 and 1982 to 2004 (Fig. 1). In the first period, the genetic gain in yield improvement was 0.77% per year, which agreed with the estimated national average (0.74%) by Meredith and Bridge (1982). However, because of the long-lasting concerted effort of four scientists including three breeders–geneticists and one agronomist in the 1980s, the breeding progress in yield improvement was accelerated and the trend has been maintained since the 1990s. The genetic gain in lint yield improvement was estimated to be 3.1% in the second period. Fiber strength has also been improved since the 1960s, which has been accompanied by steady increase in micronaire values. However, fiber length in Acala 1517 cultivars tended to decline from 30.99 to 29.98 mm from 1960 to 1990, whereas newly released Acala 1517 cultivars (Acala 1517-95, 1517-99, 1517-02, 1517-03, and 1517-04) have fiber greater than 30.48 mm. Therefore, our analysis on the statewide annual variety trials generally agrees with the previous findings for other regions. However, no yield plateau in the breeding program has been noticed. In fact, an accelerated genetic gain in yield improvement in New Mexico Acala cotton germplasm has been achieved since the early 1980s. According to the data provided by Culp and Green (1992), the number of seed per boll remained unchanged, while seed size was gradually decreased. Intuitively, this should have resulted in decrease in total seed surface area per boll for lint fiber production. Since fiber length is largely unchanged, increased lint percentage in modern cultivars was either due to more fibers per boll (or per seed) or heavier fiber (coarser) or both. On the basis of our analysis, lint percentage and micronaire value have been concurrently increased over years, whereas fiber length did not follow the same pattern. Therefore, coarser not longer fiber was the main contributing factor to higher lint percentage in the New Mexico Acala cotton germplasm improvement. Historically, obsolete Acala cotton had significantly larger bolls and seed, finer fiber, and lower lint percentage than other short staple commercial cultivars. However, the newly released high-yielding Acala cotton cultivars have relatively small boll and seed size, and high lint percentage and micronaire readings. Even though their yield potential has been substantially increased, the tendency in unintentionally reducing seed size and fiber fineness could be a concern. Efforts in cotton breeding should be taken to prevent new cultivars from further increase in micronaire.

How to increase lint yield in Acala cotton while maintaining its current high lint percentage and fiber quality
Therefore, breeding and growing Acala cotton cultivars dense fiber length beyond a certain point is negatively associated with polymorphism within Upland cotton (1–5%, Ulloa, 1989) further increase could result in a penalty in fiber pricing. It should be pointed out that we chose SSR markers (about 1/3 of the BNL SSR markers) that produced a basis of our previous studies (Zhang et al., 2005). Reducing ovule abortion can increase number of mature fibers, and short fiber content (van’t Hof, 1998) developed a technique that makes it possible to count the number of fiber cell initials on the ovules. However, a reliable and simple method in measuring fiber number is needed for practical use. Currently, the number of fibers per seed can be estimated indirectly on the basis of lint index, fiber length, and micronaire.

The earlier Acala germplasm were mainly reselections from introductions of Mexico, which did not have known germplasm introgression from G. barbadense. Interspecific hybridization with Triple Hybrid and G. barbadense including Sealand, Pima, and Tanguis was evident in perhaps the late 1930s and 1940s, and out-crossing with G. barbadense also was frequent, which resulted in Acala cotton germplasm with Verticillium wilt tolerance and better fiber quality. The SSR marker data showed that the more recently released Acala germplasm seemed to contain more common SSR markers with Pima 3-79, while they are more distant from TM-1. Thus, the limited molecular marker data support the notion based on the breeding history that Acala germplasm developed since the 1940s indeed contained genetic introgression from G. barbadense.

Another surprising note is that Acala Hopi and NM 24016 were consistently distant to other Acala germplasm (JC = 0.67–0.76). Early Acala cotton germplasm were closer to TM-1 (JC = 0.72–0.80), and Acala Original and 1064 were even grouped with TM-1. On average, the Acala cotton shared 2/3 more SSR markers with Pima 3-79 than did TM-1. Thus, the limited molecular marker data support the notion based on the breeding history that Acala germplasm developed since the 1940s indeed contained genetic introgression from G. barbadense.

It should be pointed out that we chose SSR markers (about 1/3 of the BNL SSR markers) that produced a higher level of polymorphism within Upland cotton on the basis of our previous studies (Zhang et al., 2005). One may argue that the polymorphic SSR markers used in the present study were not enough to cover the cotton genome to reliably infer the genetic relationships between Acala germplasm. Even though more than 2000 SSR primers for cotton from other sources have been developed, their full accessibility is still not free. Also, their chromosomal locations are yet revealed that would impose difficulties in marker selection for a good genome coverage. Furthermore, their extremely low level of polymorphism within Upland cotton (1–5%, Ulloa,
personal communication) suggests that obtaining adequate intraspecific polymorphic DNA markers is still a tremendous task. This explains the unavailability of even a genome-wide framework map within Upland cotton. Addition of more markers should certainly increase the reliability of genetic distance analysis. But the polymorphic SSR markers used in the present study can well discriminate the Acala germplasm and divide them into four major groups, indicating that the polymorphic markers had sufficient resolution power. One should also realize that monomorphic markers should not be discounted in genetic diversity studies since genetic distances are determined by both monomorphic (indicating similarity) and polymorphic (indicating dissimilarity) markers. The selection of the SSR markers on the known cotton chromosomes was relatively even, which should provide a framework genome coverage to determine the genetic distances among Acala germplasm. Since many BNL SSR primers did not produce polymorphism, the genetic distance obtained from the selected SSR markers was understandably higher than the tests that used randomly chosen markers. Randomly chosen DNA markers may be more accurately estimate genetic similarities if they are evenly distributed over the cotton genome. However, their genome locations are unknown in most cases because they are not mapped. Of course, the selection of markers could produce bias in overestimating the genetic diversity, but the tendency of genetic relationships between the Acala germplasm tested in the present study should remain mostly unchanged.

The Acala 1517 cotton germplasm developed from the New Mexico cotton breeding program contain desirable genes for large boll and seed size, high vigor, Verticillium wilt tolerance, and fine fiber quality. They also are most genetically diverse from other current commercial cultivars and should be promising sources in breeding to be used as parental lines to broaden genetic variations within upland cotton.

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