

# Registration of the TM-1/NM24016 Cotton Recombinant Inbred Mapping Population

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## ABSTRACT

The TM-1/NM24016 cotton (*Gossypium hirsutum* L.) mapping population (Reg. No. MP-1, NSL 477435 MAP) consists of 95 F<sub>5,9</sub> recombinant inbred lines. This cotton mapping population was constructed from a cross between inbred lines TM-1 (PI 662944 MAP), the genetic standard for *G. hirsutum*, and NM24016 (PI 662945 MAP), an advanced selection with introgressed traits from *G. barbadense* L. and *G. hirsutum*. In 2007, the population was released jointly by the Plant Physiology and Genetics Research Unit, USDA-ARS, Maricopa, AZ and New Mexico State Agricultural Experiment Station, Las Cruces, NM. The primary goal was to construct a mapping population segregating for attributes introgressed from *G. barbadense* into a *G. hirsutum* germplasm background. The TM-1/NM24016 population was genotyped with 392 simple sequence repeat markers. A wide range of phenotypic diversity among individuals was observed for fiber and agronomic traits, with transgressive variation for the majority of fiber traits. The registration of this recombinant inbred mapping population provides geneticists and breeders with an opportunity to explore the genetic basis of transgressive variation in cotton and exploit potentially novel allelic combinations for the genetic improvement of fiber quality and other traits in *G. hirsutum*.

**A**lthough the higher-yielding commercial species, *G. hirsutum* L., accounts for more than 90% of global cotton fiber production, far greater genetic potential for improved fiber length, strength, and fineness resides within *G. barbadense* L. Thus far, efforts to improve *G. hirsutum* fiber quality through introgression from *G. barbadense* have been stymied by the selective elimination of donor parent

alleles in the F<sub>2</sub> and successive generations (Stephens, 1949). As a result, there is a concomitant gradual reversion to phenotypes more similar to the *G. hirsutum* recurrent parent. In addition, advanced generations of *G. hirsutum* × *G. barbadense* populations have a marked reduction in fertility and vigor (Stephens, 1950) as well as pervasive segregation distortion (Reinisch et al., 1994; Jiang et al., 2000). These consequences of hybrid breakdown have made the recovery of desirable and stable combinations of phenotypic traits a challenging pursuit and as such have lessened the utility of interspecific breeding populations as a source of novel genetic variation for cotton breeding.

Elite *G. hirsutum* inbred lines with stabilized introgression from *G. barbadense* have been developed (Tatineni et al., 1996; Cantrell and Davis, 2000). However, the development of these cultivars was accomplished only after numerous cycles of recombination and deliberate selection for canonical phenotypes from both parental species. Such interspecific introgression lines can be employed as parents to transfer *G. barbadense* alleles to modern upland cotton breeding programs. This type of breeding approach should result in the genetic improvement of fiber quality without coincident hybrid breakdown. Beyond widening the germplasm base of *G. hirsutum*, these lines could potentially provide a window into the genetic basis of gene flow barriers between the two species. In addition, development of immortal recombinant inbred mapping populations with interspecific introgression lines could be used to dissect the genetic architecture of their unique fiber traits.

The TM-1/NM24016 cotton mapping population (Reg. No. MP-1, NSL 477435 MAP) of 95 F<sub>5,9</sub> recombinant inbred lines (RILs) was constructed to support the pursuit of

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**Abbreviations:** CV<sub>G</sub>, coefficient of genetic variation; QTL, quantitative trait locus; RIL, recombinant inbred line; SL, span length; SSR, simple-sequence repeat.

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fundamental biological questions related to interspecific introgression and to facilitate the translation of these basic research findings to cotton breeding programs. The parents of this mapping population are TM-1 (PI 662944 MAP), the genetic standard of *G. hirsutum*, and NM24016 (PI 662945 MAP), an elite *G. hirsutum* inbred line with considerable but stable introgression from diverse *G. barbadense* lines. This population has exceptional phenotypic diversity for agronomic and fiber traits (Percy et al., 2006), including physiological traits important for local adaptation (M. Gore, unpublished data, 2011) and resistance to Verticillium wilt (J. Zhang, unpublished data, 2011). These 95 RILs were genotyped with 392 simple sequence repeat (SSR) markers. The registration of this novel mapping resource will increase its accessibility to the global cotton community, which will better facilitate the genetic improvement of fiber quality in upland cotton.

## Materials and Methods

### Parentage

TM-1 (Texas Marker-1) is a homozygous inbred line that serves as the genetic and cytogenetic reference standard for upland cotton, *G. hirsutum* (Kohel et al., 1970). It was developed as a selection from 'Deltapine 14' at Texas AgriLife Research in College Station, TX.

NM24016 is an elite inbred line with the complex interspecific pedigree H12156/2/77-505/Russian 5904. It was developed through modified pedigree selection by R. Cantrell and D. Davis at the New Mexico Agricultural Experiment Station (Cantrell and Davis, 2000). Russian 5904 and 77-505 are *G. barbadense* lines, and H12156 is a white-flowered selection from an interspecific *G. hirsutum* × *G. barbadense* F<sub>3</sub> population. In the development of NM24016, there was intentional selection for traits from both parental species. Nearly two-thirds of the mosaic NM24016 genome is typical of *G. hirsutum*, based on SSR markers, while the remainder is characteristic of *G. barbadense*.

### Population Development and Evaluation

In 1994, TM-1 and NM24016 were crossed at the New Mexico Agricultural Experiment Station in Las Cruces. Several F<sub>1</sub> plants were self-pollinated to generate F<sub>2</sub> seed. An initial 118 F<sub>2</sub> individuals were advanced to the F<sub>5</sub> generation by selfing and single-boll descent without deliberate selection. Seeds of F<sub>5</sub>-derived lines were advanced by two generations of selfing and harvesting in bulk to obtain F<sub>5.7</sub>. Each of the resultant lines was derived from a single F<sub>2</sub> individual. Of the initial 118 F<sub>2</sub> individuals, only 98 F<sub>5.7</sub> RILs produced sufficient quantities of seed for a multi-environment trial (Percy et al., 2006). To replenish seed stocks of the RIL population, seeds of 95 F<sub>5.7</sub> RILs were advanced by two generations of selfing and harvesting in bulk at the Cotton Winter Nursery in Tecoman, Mexico during the winters of 2005–2006 and 2010–2011. Only 95 of the 98 RILs were registered because of the unavailability or contamination of seed sources from lines NM26, NM54, and NM59.

Field evaluations of the primary mapping population with 98 F<sub>5.7</sub> RILs were conducted under flood irrigation

conditions at Maricopa, AZ and Las Cruces, NM in 2001 and 2002. Standard agronomic and pest-control practices for irrigated cotton production in the southwestern United States were used at each location. The soil type at Las Cruces is a Glendale loam (fine-silty, mixed, superactive, calcareous, thermic Typic Torrifluvents), while in Maricopa the soil type is a Casa Grande sandy loam (fine-loamy, mixed, superactive, hyperthermic Typic Natrargids). The RIL population, TM-1, and NM24016 were planted in an RCBD with four replications at Maricopa in 2001 and 2002. Field design at Las Cruces also was an RCBD, with three replicates in 2001 and four replicates in 2002. Experimental units were two-row plots with an interrow spacing of 1.01 m at each location. Plot lengths were 12.2 m in 2001 and 10 m in 2002.

Plots were harvested mechanically with a two-row harvester. Before mechanical harvest, boll samples (50 bolls at Maricopa and 25 bolls at Las Cruces) were harvested by hand for measuring boll and fiber-quality traits. Seedcotton samples were ginned on a laboratory 10-saw gin. All of the phenotyping procedures are well described by Percy et al. (2006). Briefly, the RIL population and parents were phenotyped for ten traits: boll size, lint percentage, lint yield, plant height (only Maricopa environments), fiber length (2.5%- and 50%-span lengths), micronaire (an indirect measure of fiber fineness), strength, elongation, and uniformity. Fiber samples collected at Maricopa were analyzed by Star Lab (Knoxville, TN) with individual instrumentation, while fiber samples from Las Cruces were analyzed by a laboratory at that location with individual instrumentation. Fiber length was measured with a digital fibrograph and micronaire was measured by a Fibronaire instrument (Motion Control, Dallas, TX). Both fiber strength and elongation were measured with a stelometer.

The descriptive statistics shown in Table 1 are based only on phenotypic data collected from the 95 RILs that are included in the registration, but phenotypic data from all 98 RILs have been submitted to CottonDB (<http://cottondb.org>; verified 15 Sept. 2011).

### DNA Isolation and SSR-Marker Analysis

Self-pollinated seeds from the 95 F<sub>5.7</sub> RILs were germinated in Petri dishes lined with moistened filter paper in a growth chamber held at 32°C. Root tips (~1 cm) from an average of ten 5-d-old cotton seedlings were bulk harvested per line. Total genomic DNA was isolated from homogenized fresh 5-d-old root tissue using 2% cetyltrimethyl ammonium bromide as described by Paterson et al. (1993). Subsequently, the isolated total genomic DNA was purified with an Omega E.Z.N.A. HiBind DNA column (Omega Bio-Tek, Norcross, GA).

Parental and RIL DNA samples were genotyped with 392 SSR primer pairs as per the procedure of Fang et al. (2010). Primer sequences for markers are available from the Cotton Marker Database (<http://www.cottonmarker.org/Primer.shtml>; verified 15 Sept. 2011). Genotypic data from the two parents and 95 RILs have been submitted to CottonDB (<http://cottondb.org>).

## Characteristics

The 95 RILs and their parents were genotyped with 392 SSR markers that were chosen to collectively cover all 26 chromosomes of tetraploid cotton (Blenda et al., 2006), which lays the foundation for constructing a high-density linkage map for quantitative trait locus (QTL) analysis. However, a population size of approximately 100 RILs affords only very modest statistical power for the detection of relatively small QTL effects (Xu, 2003). Notably, residual heterozygosity was detected at 20 SSR loci in the inbred parents. Because several genetically distinct  $F_1$  plants were used to generate the initial 118  $F_2$  individuals, this residual parental heterozygosity resulted in the presence of more than two parental alleles at 15 of the 20 SSR loci in the RIL population. In addition, putative nonparental alleles were detected at 54 of the 392 SSR marker loci in the RIL population, but the overall average was only 2.5% nonparental alleles per RIL with a range of 0.8–5.4%.

The study of Percy et al. (2006) showed substantial phenotypic variability within the TM-1/NM24016 population ( $n = 98$ ), with significant genotypic variation for all measured traits. Phenotypic values of the 10 traits exhibited a continuous distribution and most of the traits had significant ( $P < 0.05$ ) genotype  $\times$  year and genotype  $\times$  location interactions. Broad-sense heritabilities were moderate to high for most traits, ranging from 0.69 for lint yield to 0.90 or greater for lint percentage, micronaire, fiber strength, and 2.5% span length (SL). In contrast, fiber elongation had a broad-sense heritability of 0.39, which is reflective of its minimal differentiation among RILs and significant ( $P < 0.0001$ ) interaction with the environment (Percy et al., 2006).

The genetic coefficient of variation ( $CV_G$ ) is the standard deviation of a trait divided by the mean of the trait (Houle, 1992). This standardization of the genetic variance provides for a more reasonable direct comparison of genetic variability among traits. In general, traits with a relatively higher  $CV_G$  will respond more favorably to selection in a breeding program (i.e., higher evolvability). Traits with the highest  $CV_G$  were plant height (65.20), boll size (14.87), and lint yield (5.28), followed by fiber length (50% SL, 4.61; 2.5% SL, 5.92), micronaire (4.33), lint percentage (3.09), and fiber strength (2.47). Fiber elongation, which had the lowest broad-sense heritability, had the second lowest  $CV_G$  (1.66) of all traits, followed by fiber uniformity with a  $CV_G$  of 1.25.

Transgressive variation typically is exhibited in segregating populations that are constructed from interspecific crosses (Tanksley, 1993). As reflected by the parental means and RIL ranges shown in Table 1, the TM-1/NM24016 population ( $n = 95$ ) captured remarkable levels of transgressive variation, especially for fiber traits. While nearly 30% of the population possessed fiber strength equivalent ( $\alpha = 0.05$ ) to that of the higher parent, NM24016 (230.1 kN m kg<sup>-1</sup>), four

**Table 1. Means and ranges for traits evaluated within the TM-1/NM24016 RIL population ( $n = 95$ ) and midparent values in four summer environments: Las Cruces, NM and Maricopa, AZ across 2 yr.**

Traits	RIL population		Parent means		
	Mean	Range	NM24016	TM-1	Midparent
Boll size (g boll <sup>-1</sup> )	4.8	4.1–6.0	4.4	6.1	5.2
Lint percentage (%)	34.0	30.0–40.0	36.1	33.4	34.7
Lint yield (kg ha <sup>-1</sup> )	1139.9	870.3–1593.8	1015.2	1358.7	1186.9
Plant height (m)	1.2	0.9–1.5	1.1	1.0	1.1
Micronaire (unit)	4.4	3.6–5.0	4.3	4.9	4.6
Fiber elongation (%)	6.3	4.9–7.4	5.8	6.4	6.1
Fiber strength (kN m kg <sup>-1</sup> )	217.5	196.2–255.3	230.1	201.9	216.0
50%-span length (mm)	14.6	13.6–16.1	15.1	14.5	14.8
2.5%-span length (mm)	30.7	27.9–33.8	31.7	30.1	30.9
Length uniformity (%)	47.7	44.3–50.8	47.6	48.2	47.9

transgressive RILs had fiber strength in excess of 240 kN m kg<sup>-1</sup>. Similarly, about 25% of the RILs had a 2.5% SL nearly identical to or greater ( $\alpha = 0.05$ ) than the longer-fibered NM24016 (31.7 mm), but almost 15% of the RILs exhibited transgressive variation by having a shorter ( $\alpha = 0.05$ ) fiber length than TM-1 (30.1 mm). In addition, approximately 15% of the RILs had micronaire values less ( $\alpha = 0.05$ ) than the lower parent, NM24016 (4.3 units), a highly desirable characteristic in mature fiber of upland cotton. Given that lower micronaire is preferred, the negative phenotypic correlation ( $r = -0.47$ ) between micronaire and 2.5% SL permits the simultaneous genetic improvement of these two fiber traits.

## Availability

Phenotypic and marker data for the TM-1/NM24016 population have been submitted to the CottonDB database (<http://cottondb.org>). Seeds of the TM-1/NM24016 mapping population and parents have been deposited in the National Center for Genetic Resources Preservation at Fort Collins, CO and the USDA-ARS National Cotton Collection in College Station, Texas. Contact the corresponding authors for all seed requests, including the parental seed sources that were genotyped. Small quantities of seed for research purposes will be available for distribution from the corresponding authors for 5 yr after the date of this publication. Beyond 5 yr after the date of this publication, all seed requests should be made to the USDA-ARS National Cotton Collection. Seed requests from outside the USA must be accompanied by an import permit allowing entry into the requestor's country. The provider may not be able to certify that seed is free of certain insects or pathogens specified on import permits, and in such cases seed cannot be supplied. Should this material contribute to further research or germplasm development, we request that the source of this mapping population be acknowledged by citing this registration.

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