

## Four Chromosome-Specific (*Gossypium barbadense* Chromosome 5sh) Upland Cotton RILs with Improved Elongation

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### Abstract

A chromosome-specific recombinant inbred line (CS-B05shRIL) population was created from a cross of TM-1, the genetic standard line of *Gossypium hirsutum* L., and CS-B05sh, a previously released interspecific chromosome substitution line in which all of the chromosome pairs are genetically similar to those of TM-1, except for the short arm of chromosome 5, which is substituted from 3-79 (*G. barbadense* L.). Four of the fifty CS-B05shRILs were selected and released on the basis of their improved elongation ranging from 7.37 to 7.84%. The four selected RILs are identified as CS-B05shRIL-93 (Reg. No. GP-1014, PI 677339), CS-B05shRIL-68 (Reg. No. GP-1013, PI 677337), CS-B05shRIL-66 (Reg. No. GP-1012, PI 677336), and CS-B05shRIL-10 (Reg. No. GP-1011, PI 677334). The fiber properties among the CS-B05shRILs were compared with those of commercial cultivars DP 393 and PHY 370 WR, which had elongations of 6.86 and 6.25%. Commercial lines performed better for agronomic traits than the released lines. Fiber elongation (the ability to stretch before breaking) is a critical trait in determining yarn quality. The combination of strength and elongation in a fiber determines the energy needed to break either a fiber or a yarn. Due to changes in textile technologies, the global market demands cotton cultivars with improved potential for tensile properties. These germplasm lines are released to incorporate improved elongation genes from only the short arm of chromosome 5 of *G. barbadense*, thereby reducing genetic drag effect compared with the conventional method of interspecific introgression involving the whole genome.

THE USDA-ARS Mississippi Agriculture and Forestry Experiment Station and Texas A&M AgriLife Research developed and jointly released four germplasm lines of upland cotton (*Gossypium hirsutum* L.) as CS-B05shRIL-93 (Reg. No. GP-1014, PI 677339), CS-B05shRIL-68 (Reg. No. GP-1013, PI 677337), CS-B05shRIL-66 (Reg. No. GP-1012, PI 677336), and CS-B05shRIL-10 (Reg. No. GP-1011, PI 677334), which have excellent elongations of 7.64, 7.84, 7.37, and 7.66%, respectively, under a range of growing environments (Table 1). The fiber properties among the CS-B05shRILs were compared with those of commercial cultivars DP 393 (PI 635100) and PhytoGen brand PHY 370 WR (Saha et al., 2012), which had elongations of 6.86 and 6.25%, respectively. However, agronomic traits of both commercial lines are better than those of the released lines.

These materials are genetically similar to 'TM-1' (PI 674746) the genetic standard line of upland cotton (Kohel et al., 2001); however, each differs by the replacement of a specific segment of homologous pairs of the chromosome 5 short arm from 3-79, a *G. barbadense* genetic standard. TM-1 is a highly inbred line derived from the commercial cultivar Deltapine 14 (PI 528970). Line 3-79, the donor parent for the substituted segment of the chromosome 5 short arm in the released lines, originated as a doubled haploid from Pima germplasm with higher fiber quality than modern Pima germplasm (Endrizzi et al., 1985). An integrated approach of conventional breeding and cytogenetic method was used to develop four chromosome-specific RILs (CS-B05shRIL) carrying a different segment of the short arm of *G. barbadense* chromosome 5 substituted for the respective segment of the *G. hirsutum* chromosome 5 short arm with improved elongation. It is difficult to introgress genes from alien tetraploid *Gossypium* species into *G. hirsutum* because of genetic incompatibility. However, we used chromosome substitution lines as the donor parent (Stelly et al., 2005) to reduce genetic incompatibility and specifically increase recombination of the substituted chromosome segment. The four lines provide a scope of the facility and feasibility of transferring desirable traits from *G. barbadense* with reduced linkage drag effects.

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**Abbreviations:** RIL, recombinant inbred line.

**Table 1. Mean values of the fiber trait for the released cotton recombinant inbred lines (RILs) and commercial cultivars grown in 2008 and 2009 in two different locations at Mississippi State University and one location at Florence, SC. Data were extracted from large multiple entry tests.**

Name	Boll weight	Lint	Seed yield	Lint yield	Uniformity index	Strength	Micronaire	UHM†	Elongation
	g	%	kg ha <sup>-1</sup>			kN m kg <sup>-1</sup>		mm	%
CS-B05shRIL-93	4.54	33.23	1705	569	83.56	256.62	4.40	28.09	7.64
CS-B05sh-RIL-68	4.08	33.66	1669	563	83.47	272.51	4.92	27.86	7.84
CS-B05shRIL-66	4.95	33.68	1821	617	83.78	266.13	4.54	28.22	7.37
CS-B05shRIL-10	4.74	33.29	1711	574	83.63	261.43	4.59	28.09	7.66
DP 393	4.87	42.44	2079	885	84.32	291.63	4.54	29.59	6.86
PHY 370 WR	5.02	43.95	2502	1097	82.96	282.51	4.59	27.61	6.25
Entry × env F‡	*	*	*	*	ns§	*	ns	ns	*
Entry F¶	*	*	*	*	*	*	*	*	*
LSD <sub>0.05</sub>	0.27	0.56	334	116	0.62	6.96	0.25	0.51	0.28
Average of CS-B05shRILs#	4.9	33.48	1659	562	83.57	276.24	4.69	28.45	6.83

\* Significant at the 0.05 probability level.

† UHM, upper-half mean fiber length.

‡ Individual line and environment interaction.

§ ns, not significant.

¶ Individual line.

# Average of 50CS-B05shRIL population from which these four CS-B05shRILs were selected.

## Methods

The released lines were developed from a cross of TM-1 and CS-B05sh (Stelly et al., 2005), a formally released interspecific chromosome substitution line in which all of the chromosome pairs are genetically similar to TM-1, except for the pair of chromosome 5 short-arm segment. The chromosome 5 short-arm homolog pair in CS-B05sh has been substituted from *G. barbadense* line 3-79. These lines were produced by crossing TM-1 with CS-B05sh to produce F<sub>1</sub> seeds; TM-1/CS-B05sh F<sub>2</sub> seeds were produced in 2004 by self-pollination of F<sub>1</sub> plants at a winter nursery at Tecoman, Mexico. Fifty individual plants of the F<sub>2</sub> population were inbred by self-pollination for five generations using the single-seed decent method and were then self-pollinated to increase seeds to establish a population of *G. barbadense* chromosome-5sh-specific RILs (CS-B05shRILs). In 2008 and 2009, the population of 50 individual CS-B05shRILs were grown in two locations at Mississippi State University, Mississippi State (33.4° N, 88.8° W), with the commercial cultivars DP 393 and PHY 370 WR and one location at Florence, SC (34.1° N, 79.4° W). Soil types at Mississippi State were a Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquept; Location 1) and Marietta loam (fine-loamy, siliceous, active, fluvaquent Eutrudepts; Location 2). The soil type at Florence was a Norfolk loamy sand (fine-loamy, kaolinitic, thermic tykpid Kandiodults; Location 3). Lines of the CS-B05shRIL population were evaluated by a randomized complete block design with four replications within each environment. Standard agronomic practices were followed during the growing season for all environments. Each entry was grown in single-row plots 12 m long with rows spaced 97 cm and plants spaced 10 cm apart (about 110 plants row<sup>-1</sup>). A 25-boll sample per plot was hand harvested from the first fruited positions at the middle nodes of plants to determine fiber properties. Samples were ginned on a 10-saw laboratory gin to determine lint percentage. The lint and seed fractions were weighed and lint percentages were calculated by dividing lint fraction by total weight of lint and seed, then multiplying by

100. The lint samples were sent to Cotton Incorporated's fiber lab for high volume instrument fiber quality trait measurement. After the boll samples were harvested, all plots were harvested with a commercial cotton picker modified to bag seed cotton from each plot. On the basis of this weight, lint yield per plot was calculated. The average value for each agronomic and fiber quality trait of individual CS-B05shRILs was calculated. Data were subjected to ANOVA using SAS version 8.0 (SAS Institute, 1999). Means were separated using Fishers protected LSD at the 0.05 probability level. Four of fifty CS-B05shRILs (CS-B05shRIL-93, CS-B05shRIL-68, CS-B05shRIL-66, and CS-B05shRIL-10) were selected for release from the CS-B05shRIL population on the basis of their superior elongation. The released lines had poor agronomic quality compared with commercial cultivars of DP 393 and PHY 370 WR because both of the parents (TM-1 and 3-79) had poor agronomic qualities compared with the commercial cultivars. The primary objective of this research was to use a novel method for targeted introgression of improved fiber quality traits from *G. barbadense* to reduce linkage drag effects and other problems associated with the conventional method of interspecific introgression between the two species (Endrizzi et al., 1985). The germplasm lines being released were developed and evaluated in a collaborative research program of the USDA-ARS Crop Science Research Laboratory, Mississippi State University, the USDA-ARS Coastal Plain, Soil, Water, and Plant Research Center, and Texas A&M AgriLife Research.

## Characteristics

Four of the fifty CS-B05shRILs were selected for release on the basis of their improved elongation ranging from 7.26 to 7.84% (Table 1). All of these lines showed improved elongation compared with commercial cultivars DP 393 and PHY 370 WR, which had elongations of 6.86 and 6.25%, respectively. The selected CS-B05shRILs had also greater elongation than the recurrent parent TM-1, 3-79, and CS-B05sh, which were grown in the same locations and year in a separate experiment (Table 2). These germplasm lines are released to incorporate genes

**Table 2.** Mean values of the fiber trait for TM-1, 3-79, and CS-b05sh (the parental cotton lines) grown in 2008 and 2009 in the same two locations at Mississippi State University and one location at Florence, SC, where the released CS-B05sh recombinant inbred lines were grown, but in a separate experiment. Data were extracted from large multiple entry tests in the same year and locations as a separate experiment.

Name	Boll weight	Lint	Seed yield	Lint yield	Uniformity index	Strength	Micronaire	UHM†	Elongation
	g	%	kg ha <sup>-1</sup>			kN m kg <sup>-1</sup>		mm	%
TM-1	5.47	34.30	1894	657	83.66	283.98	4.75	28.78	6.57
3-79	3.14	33.65	657	212	86.09	369.10	3.63	34.95	6.58
CS-B05sh	4.49	36.08	1592	582	82.35	266.53	4.87	27.66	6.17
Entry × env F‡	*	*	*	*	*	*	*	ns	*
Entry F§	*	*	*	*	*	*	*	*	*
LSD <sub>0.05</sub>	0.19	0.56	337	140	0.48	8.73	0.16	0.51	0.17

\* Significant at the 0.05 probability level.

† UHM, upper-half mean fiber length.

‡ Individual line and environment interaction.

§ Individual line.

especially for elongation from *G. barbadense*. Breeders typically rely on a whole-genome strategy in conventional breeding methods of interspecific introgression with *G. barbadense*. Such strategies often result in progenies containing a large number of alien genes associated with undesirable traits as a result of linkage drag. The extent of linkage drag is greatly reduced, compared with the conventional method of interspecific introgression, when a homolog pair of a segment from the donor alien species in a single-chromosome RIL is used for interspecific introgression of improved traits. The single-chromosome RILs provide genes to improve elongation from *G. barbadense* with reduced effects of linkage drag. It is important to note that, when TM-1 was crossed with CS-B05sh, the resulting TM-1/CS-B05sh F<sub>1</sub> hybrids were highly heterozygous for the short arm of chromosome 5 but were completely or almost completely homozygous for all other chromosomes of the *G. hirsutum* TM-1 complement. In meiosis of the TM-1/CS-B05sh F<sub>1</sub> hybrids, the substituted short-arm segment of chromosome 5 from *G. barbadense* was only expected to recombine with the respective homologous *G. hirsutum* chromosome segment and to segregate in the F<sub>2</sub> generation. For the other chromosomes (non-5sh), the amount of alien genetic material in CS-B05sh is expected to be low or none (theoretical average 1.5625%) because CS-B05sh was developed using the hypoaneuploid-based modified backcrossing method to the BC<sub>5</sub>F<sub>1</sub> generation and then inbred (Stelly et al., 2005), so the CS-B05shRILs segregated only for the genes located on the short arm of chromosome 5.

The lines being released were selected for greater elongation, an improved fiber quality trait, from 50 CS-B05shRILs on the basis of the results of 2-yr field trials at two different locations in diverse environments. Fiber elongation (the ability to stretch before breaking) is one of the important traits in determining yarn quality. Producers prefer cotton cultivars with improved potential for tensile properties. These lines, carrying a small segment of the chromosome 5 short arm from *G. barbadense*, provide an effective method for upland cotton improvement by targeted interspecific introgression of elongation, a desirable trait from *G. barbadense* with reduced linkage drag effects.

## Availability

Small amounts of self-pollinated seed (S<sub>c</sub>) are available for distribution to cotton geneticists, breeders, and other research personnel on written request to Dr. S. Saha, USDA-ARS, PO Box 5367, Mississippi State, MS 39762. Genetic material of this release will be deposited in the National Plant Germplasm System, where it will be available immediately for research purposes, including development and commercialization of new material. It is requested that appropriate recognition of the source be given when the germplasm lines contribute to research or the development of improved lines, cultivars, or hybrids.

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