Evaluation of a Chromosome Segment from *Gossypium barbadense* Harboring the Fiber Length QTL *qFL-Chr.25* in Four Diverse Upland Cotton Genetic Backgrounds

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ABSTRACT

Competition with manmade fibers has increased focus on upland cotton (*Gossypium hirsutum* L.) fiber quality, especially upper half mean length (FL), for industry stakeholders and breeders. The purpose of this study was to characterize the effect of *qFL-Chr.25*, a quantitative trait locus (QTL) introgressed from *G. barbadense* L. originally identified in 'Sealand 883'. The QTL was deployed within four genetic backgrounds: Acala SJ4, Paymaster HS26, Deltapine 50 (DP 50), and Georgia 2004089 (GA089), each representing the gene pools of the major US cotton-growing regions: the US Southwest, Texas High Plains, Mississippi Delta, and US Southeast, respectively. In highly related bulked sister lines (BSLs), the effect of *qFL-Chr.25* was significant in the DP 50 (1.4 mm) and GA089 (1 mm) backgrounds in trials grown in Tifton, GA, in 2014 and 2015. In multilocation trials planted across the Cotton Belt in 2011 of 25 lines from each of the four backgrounds carrying the *G. barbadense* allele and 25 without the introgressed allele, FL of the QTL(+) lines were numerically higher than the QTL(–) lines by 0.9 mm, though not statistically significant. Recombinant genotypes recovered from the Paymaster HS26 and GA089 backgrounds, and development of additional simple sequence repeat (SSR) markers within the QTL region, allowed further refinement of the *qFL-Chr.25* region's boundaries from ~2.2 to 0.8 Mb. Although variable effects were seen from this QTL, observation in a more isogenic state such as the BSLs and recombinant lines made the effect of the QTL more apparent. Incorporation of this QTL into breeding programs aided by these newly developed SSR markers should help in the utilization of this introgression to improve cotton fiber quality.

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Abbreviations: BSL, bulked sister line; DP 50, Deltapine 50; ELO, elongation; FL, upper half mean length; GA089, Georgia 2004089; HVI, high volume instrument; IP, individual plant; LP, lint percentage; MIC, micronaire; PM HS26, Paymaster HS26; QTL, quantitative trait locus/loci; QTL(+), lines with Sealand 883 background and the Sealand 883 allele at *qFL-Chr.25*; QTL(–), lines with Sealand 883 background without the Sealand 883 allele at *qFL-Chr.25*; PCR, polymerase chain reaction; SFC, short fiber content; SL883, Sealand 883; SSR, simple sequence repeat; STR, fiber bundle strength; UI, uniformity index.

The quality of lint fibers produced by upland cotton (*Gossypium hirsutum* L.) is a major focus of the cotton industry. Market demands from textile mills seeking long, strong, and uniform cotton fiber have prompted cotton researchers to develop cultivars with improved fiber quality and further refine production practices that maximize genetic potential for these quality traits. Today, a major component of international trade for US cotton is the Asian yarn and textile markets. Increased competition from manmade fibers to satisfy market demands from the

Published in Crop Sci. 59:2621–2633 (2019).
doi: 10.2135/cropsci2019.05.0321

mills and fashion industry is placing ever more importance on the improvement of cotton fiber quality.

Fiber quality of all cotton bales produced within the United States are analyzed with a high volume instrument (HVI) to assess multiple fiber quality parameters. Fiber length is commonly measured by HVI as upper half mean length (FL), which is the average length of the longest half of fibers in a sample. This measure is the industry standard for assessing fiber length and is a critical quality to textile spinners. Although incremental gains have been achieved for FL, those gains have been small and have shown signs of periodic stagnation (Helms, 2000; Lewis, 2001; May et al., 1995; Meredith et al., 1997). It is assumed that stagnation in fiber quality improvement is due to lack of genetic diversity within the crop (Esbroeck and Bowman, 1998). To increase the genetic diversity within upland cotton germplasm, an appealing strategy is to introgress fiber traits from related species such as *Gossypium barbadense* L., the domesticated forms of which are also known as Egyptian, sea island, and pima cotton. However, interspecific introgression of *G. barbadense* into the more productive species, *G. hirsutum*, often results in deleterious effects such as hybrid breakdown (Stephens, 1949), sterility, and abnormal segregation in the progeny (Jiang et al., 2000; Reinisch et al., 1994). These factors have made interspecific introgressions difficult to incorporate into elite or commercial germplasm, though some successful cases involving extensive backcrossing efforts have been documented (Campbell et al., 2010, 2011; Cantrell and Davis, 1993; Knight and Hutchinson, 1950; Tatineni et al., 1996). A useful strategy to incorporate some of these potentially beneficial alleles from *G. barbadense* has been to use introgression lines with portions of *G. barbadense* genome in a primarily *G. hirsutum* genetic background (Percy et al., 2006; Shen et al., 2007; Sun et al., 2012; Zeng and Meredith, 2009; Zhang et al., 2012; Zhang et al., 2009).

Numerous fiber quality quantitative trait loci (QTL) have been mapped in interspecific populations or introgression lines derived from *G. barbadense* (Jiang et al., 2000; Chen et al., 2009; Draye et al., 2005; Lacape et al., 2003; Paterson et al., 2003; Shen et al., 2007; Wang et al., 2011; Zhang et al., 2014; Zhang et al., 2009), yet few have been evaluated in multiple genetic backgrounds and environments. When a QTL of interspecific origin is introgressed into adapted germplasm, unexpected interactions with genetic background have been observed in soybean [*Glycine max* (L.) Merr.] and tomato (*Solanum lycopersicum* L.) (Bernacchi et al., 1998; Reyna and Sneller, 2001), including varied or total lack of effect in given backgrounds, unintended impacts on other traits, or even lack of expression in different environments. In upland cotton, Chee et al. (2005a, 2005b) and Draye et al. (2005) reported that genetic backgrounds can have profound interactions with effects of QTL for fiber length, fineness, and elongation (ELO). For this reason, it is critical that introgressed QTL segment(s) be evaluated in varying genetic backgrounds and target environments, before extensive marker-assisted breeding efforts are undertaken.

One of the oldest public US cotton breeding programs, the USDA-ARS's Pee Dee breeding program in Florence, SC, used interspecific hybridization extensively starting in the 1940s and continuing into the 1970s to improve *G. hirsutum* fiber quality. An example of their use of interspecific introgression to improve fiber quality is Beasley's Triple Hybrid, a germplasm line derived from a cross between *G. arboreum* L., *G. thurberi* Todaro, and *G. hirsutum*. This germplasm line has been widely credited for positively influencing fiber bundle strength (STR) of much of the US cotton germplasm. For example, Beasley's Triple Hybrid can be found in the foundational pedigrees of the New Mexico Acala and Pee Dee breeding programs (Smith et al., 1999), both of which have been important to the improvement of upland cotton. More recently there have been fiber quality alleles introgressed from *G. arboreum* (Sun et al., 2012), *G. tomentosum* Nutt. ex Seem. (Zhang et al., 2011), *G. mustelinum* Miers ex Watt (Wang et al., 2016), and *G. klotzchianum* Anders (Xu et al., 2012) with significant effects.

Among some of the germplasm developed through interspecific hybridization by the Pee Dee breeding program were the "Sealand" lines. These germplasm lines were the result of a cross between 'Bleak Hall', a sea island cultivar (*G. barbadense*) with extra-long staple fiber, and 'Coker Wilds', a *G. hirsutum* cultivar (Bowman, 2006; Culp and Harrell, 1974). The Sealand lines were considered *G. hirsutum* upland cultivars with excellent FL and STR but were never grown extensively on a commercial scale. Previous research indicated that many of the introgressed segments from *G. barbadense* within the Sealand lines harbored important QTL for STR and FL (Kumar et al., 2012, 2019). One of these QTL, *qFL-Chr.25*, had a large effect, explaining 4.4 to 61.4% of the variation in FL in the original mapping populations, and was mapped to a 7 cM region delineated by simple sequence repeat (SSR) markers BNL827 and NAU2714 (Kumar et al., 2019).

Cotton breeding in the 20th century saw a more concerted effort to produce cultivars adapted to the various macro-environments of the US Cotton Belt such as the acala, plains, delta, and eastern types (Meredith, 1991; Niles and Feaster, 1984). Acala-type cultivars are grown primarily west of Texas, such as New Mexico and California. They are largely based on stocks from Acala and Tuxtla, Mexico (Chiapas), with some degree of influence from Beasley's Triple Hybrid, as well as some significant *G. barbadense* germplasm introgression (Meredith, 1991; Niles and Feaster, 1984; Ware, 1951). In North and West Texas, the plains-type cultivars were developed, which

are distinguished by their short stature, early-season maturation, and tight, wind-resistant boll. These cultivars were largely derived from a cultivar named 'Big Boll Stormproof' collected in Mexico in the 1850s (Niles and Feaster, 1984; Ware, 1951). The delta-type cultivars, grown primarily in the Mississippi Delta but adaptable Beltwide, were developed from pre-1900 cultivars such as 'Lone Star', which was derived from some 1860 and 1880 introductions from Mexico (Ramey, 1966; Ware, 1951). Eastern cultivars were largely made up of Coker germplasm, which includes many selections from the 19th and 20th century Mexican introductions, including some possible *G. barbadense* introgression (Niles and Feaster, 1984). The cottons grown in these macro-environments show significant phenotypic diversity; however, DNA analysis using SSR and single nucleotide polymorphism (SNP) markers have shown a considerable degree of genetic similarity (Ai et al., 2017; Fang et al., 2013).

This study (i) determined the effect of *qFL-Chr.25* in highly related sister lines with reduced extraneous segregation, (ii) examined the variable effect of *qFL-Chr.25* in four genetic backgrounds adapted to US growing regions and estimated genotype × environment interactions, and (iii) identified additional recombinant individuals to refine the *qFL-Chr.25* interval.

MATERIALS AND METHODS

The lines developed for this study were derived from crosses of 'Sealand 883' (SL883, PI 528875) with four regionally adapted cultivar parents, 'Acala SJ4' (PI 529538), 'Paymaster HS26' (PM HS26, PI 606814), 'Deltapine 50' (DP 50, PI 529566), and an unreleased breeding line from the University of Georgia Molecular Cotton Breeding Laboratory, Georgia 2004089 (GA089). An obsolete cultivar, SL883 was released in 1945 by the USDA Pee Dee Cotton Breeding program in South Carolina resulting from the cross of a *G. hirsutum* parent, Coker Wilds, with Bleak Hall, a *G. barbadense* sea island cotton cultivar (Bowman, 2006; Culp and Harrell, 1974). The resulting progeny were backcrossed several times to Coker Wilds to create the Sealand germplasm lines, including SL883, which is an upland cotton with excellent fiber quality, especially fiber length, due in part to significant *G. barbadense* introgression (Bowman, 2006; Culp and Harrell, 1974). Three experiments were performed to characterize the effect of *qFL-Chr.25* and are presented here as subsections.

Development and Assessment of Bulked Sister Lines

Bulked sister lines (BSLs) were selected for the presence [QTL(+)] or absence [QTL(−)] of SL883 genome targeting the *qFL-Chr.25* region to mimic the properties of nearly isogenic lines to clearly define the effect of the QTL. Sealand 883 was crossed with Acala SJ4, PM HS26, DP 50, and GA089 to create independent lineages. In 2008, genetic analysis (using markers NAU2713 and CIR109) of individual F_3 plants within each lineage identified plants based on their heterozygosity for the genomic region containing *qFL-Chr.25*. Seed was harvested from heterozygous F_3 individuals and used to plant 348 segregating F_4 nursery rows for seed increase in 2011. Eighty-nine F_4 nursery rows were grown from the Acala SJ4 background, 81 were grown from the DP50 background, 88 were grown from the PMHS26 background, and 90 were grown from the GA089 background. All segregating F_4 nursery rows were individually bulk harvested.

Bulk-harvested $F_{3:4}$ nursery row seed was used to plant 15 randomly selected $F_{3:5}$ segregating lines, or subfamilies from within each of the four lineages. Planted in the greenhouse in winter 2013, 60 total lines were genotyped for the presence [QTL(+)] or absence [QTL(−)] of the genomic region spanning *qFL-Chr.25*. At least 22 individual plants (IPs) per segregating subfamily were genotyped using SSR markers that spanned the ∼7 cM region of interest. Genomic DNA extraction followed a published protocol (Paterson et al., 1993), and polymerase chain reaction (PCR) amplification conditions were modified from methods described by Chee et al. (2004). The 10-μL PCR reactions contained approximately 10 ng of DNA, 0.5 μM of forward and reverse SSR primers, 100 μdNTPs, 1.5 mM $MgCl_2$, 3 U of DNA taq polymerase, and 1× reaction buffer containing 100 mM Tris-HCl and 500 mM KCl at pH 8.3. The PCR thermal cycling conditions were 94°C for 3 min, then 34 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 1 min, then incubation at 72°C for 7 min, prior to cooling down for storage at 12°C. The PCR products were visualized using 10% polyacrylamide gel electrophoresis and staining with silver nitrate similarly to previously published procedures (Zhang et al., 2002).

Five SSR markers that span the ∼7 cM region (BNL827, BNL3359, CIR109, CIR267, and CIR 298) were chosen to genotype IPs, all of which were identified during the earlier mapping study to be polymorphic between SL883 and at least one of the four regionally adapted cultivars (Kumar et al., 2019). These five SSR markers were used for genotyping to mitigate potential recombination that could lead to false positive or negative assessment of the *qFL-Chr.25* region. To construct the BSLs, four homozygous plants per $F_{3:5}$ line were selected for the presence [QTL(+)] or absence [QTL(−)] of SL883 genome targeting the *qFL-Chr.25* region from each of the subfamilies. Not all of the subfamilies were segregating as expected in the small subsample of plants genotyped, and thus not all of the 15 tested subfamilies per cultivar lineage produced BSLs for this experiment. Sister lines were bulk harvested together to serve as seed source of the homozygous QTL(+) and QTL(−) BSLs for each family lineage. From the SL883 × Acala SJ4 population, there were five segregating subfamilies from which BSLs were extracted; this led to five QTL(+) BSLs, and five corresponding QTL(-) lines, for a total of 10 lines from the SL883 × Acala SJ4 population. Similarly, the SL883 × PM HS26 lines consisted of 12 BSLs—six that were QTL(+), and six QTL(−). The SL883 × DP 50 BSLs were made up of 19 BSLs—nine QTL(+) lines and 10 QTL(−) lines; one of the QTL(+) lines did not survive. The SL883 × GA089 BSLs consisted of 28 total lines, 14 QTL(+), and 14 QTL(−). Altogether, there were a total of 69 BSLs advanced to field trials, 34 of which were QTL(+), and 35 of which were QTL(−).

In 2014 and 2015, the BSLs from each family lineage were grown in Tifton, GA, in a randomized complete block design with two replications along with SL883 and the regional cultivar parents (Acala SJ4, PM HS26, DP 50, and GA089) as checks. In 2014, the plots were single rows spaced 1 m apart and 3.3 m long. In 2015, the plots were two rows wide, spaced 1 m apart, and 10 m long. A 30-boll sample was harvested from the mid-fruiting zone of the plants to represent the genetic potential for fiber quality and provide uniformity within the sample. These 30-boll samples were ginned on laboratory 10-saw gins in 2014, and on 20-saw tabletop gins in 2015 to determine lint percentage (LP). The resulting fiber was analyzed with a HVI by the Fiber Quality Laboratory at Cotton Incorporated in Cary, NC. The HVI fiber parameters measured included micronaire (MIC), FL, uniformity index (UI), STR, ELO, and short fiber content (SFC). All measured HVI properties are discussed in this section to give context of the material.

Statistical analyses were conducted using SAS/STAT Software, version 9.4 (SAS Institute, 2014). The PROC GLM function was used for analyses of variance and mean separations. Waller–Duncan's method for mean separation was chosen as a more conservative approach for separating means when *F* values are low, and similarly conservative to Fisher's when *F* values are high.

Genotype × Environment Interaction of *qFL-Chr.25*

Lines used for this experiment were derived from the F_3 population discussed previously; however, for this experiment a total of 50 IPs, 25 of which were homozygous QTL(+) and 25 of which were homozygous QTL(−), were selected from each of the four cultivar parent genetic background families, for a total of 200 lines. The lines were selected at random based on the presence or absence of the SL883 allele using the nearby SSR markers CIR267 and BNL827. At the time this project was initiated, these markers were thought to be very tightly linked. Further refinement of the QTL interval after field trials were conducted indicated that CIR267 and BNL827 were not as tightly linked to the QTL as previously believed and could potentially recombine away from *qFL-Chr.25*. This could be a possible explanation for the limited effect of the QTL in these field trials.

After a progeny row seed increase in 2010, replicated yield trials were conducted across the Cotton Belt of the $F_{3:5}$ and $F_{3:6}$ lines in 2011 and 2012, respectively. Trials were conducted at the University of Arizona, Maricopa, AZ; Texas Tech University, Lubbock, TX; Louisiana State University, Alexandria, LA; and Clemson University's Pee Dee Research and Education Center, Florence, SC. Field trials were arranged in a randomized complete block design with two replications at each site. Trial plots were single-row plots, ∼6 m in length, with rows spaced 1 m apart. Regional best growing practices were followed where these trials were conducted. The objective of this experiment was to demonstrate the effect of genetic background, background × location, QTL, QTL × background, QTL × location, and QTL × background × location. To achieve this, 2 yr of data from each of the locations would have been ideal; however, 2012 trial data had to be discarded due to a seed increase harvest mix-up in 2011. Thus, statistical power was diminished to such a degree that teasing apart the expected differences and interactions could not be realized as intended with only a single year's data.

Boll samples were harvested from the mid-fruiting zone from each plot to represent the best bolls on the plant and to ensure a uniform sample. Twenty-five bolls were hand harvested from each plot and were ginned on a laboratory style 10-saw gin. Fiber samples were sent to Cotton Incorporated's Fiber Quality Testing Laboratory in Cary, NC, for HVI analysis. Measured HVI fiber parameters included those mentioned previously, although FL will be the focus of this discussion. Statistical analyses were conducted as previously described.

Refining the *qFL-Chr.25* Interval

Segregating F_4 lines from each genetic background were screened for recombination within the previously delineated region of *qFL-Chr.25* spanning ∼7 cM (Kumar et al., 2019), which is equivalent to 2.15 Mb based on marker physical positions in the *G. raimondii* Ulbrich sequence (Paterson et al., 2012). Of the 1185 F_4 plants genotyped, two were confirmed as heterozygous recombinants within the QTL region, one from the PM HS26 background (plant identification number 1066), and one from the GA089 background (plant identification number 5064) (Fig. 1). F_5 progeny from these recombinant plants were increased in the greenhouse. The two families were advanced to the F_7 generation in 2013, from which individual heterozygous plants were harvested for further testing. Heterozygosity was retained through generations of selfing to ensure the QTL region would remain heterozygous to encourage recombination and that background genome would become highly homozygous to reduce the opportunity of nontarget segregation obscuring the effect of the QTL region on FL.

Using the reference *G. raimondii* genome sequence (Paterson et al., 2012), additional SSR markers were developed to further saturate the *qFL-Chr.25* region. Sixteen PCR primers were developed (Operon), with four (UGT2501, UGT2504, UGT2509, and UGT2516) showing reliable amplification and polymorphism between parents (Supplemental Table S1). Amplification of PCR products was performed as described in Chee et al. (2004), electrophoretically separated using 10% nondenaturing polyacrylamide gel electrophoresis, and visualized by staining with silver nitrate as described (Zhang et al., 2002).

In 2014, IPs grown in the field in Tifton, GA, were tagged and DNA was extracted from each of them. The populations were screened using a representative panel of SSR markers spanning the region of interest, UGT2501, UGT2504, CIR298, UGT2509, CIR267, UGT2516, and BNL827. Individual plants from the three genetic classes within these families were harvested and ginned. The populations derived from plants 1066-73, 1066-77, 1066-93, and 5064-418 were only grown in 2014. The populations derived from 1066-78, 5064-422, and 5064-440, however, were tested as IPs in 2014 and replicated progeny row plots in 2015. In 2015, seeds from the IPs harvested in 2014 were used to plant the replicated progeny trial of these lines. If the QTL was located within the delineated region, the effect would be identifiable in resulting progeny rows. Plots were single rows, spaced 1 m apart, and were ∼3.3 m long with two replications. Representative 30-boll samples were harvested from the mid-fruiting zone within the progeny row

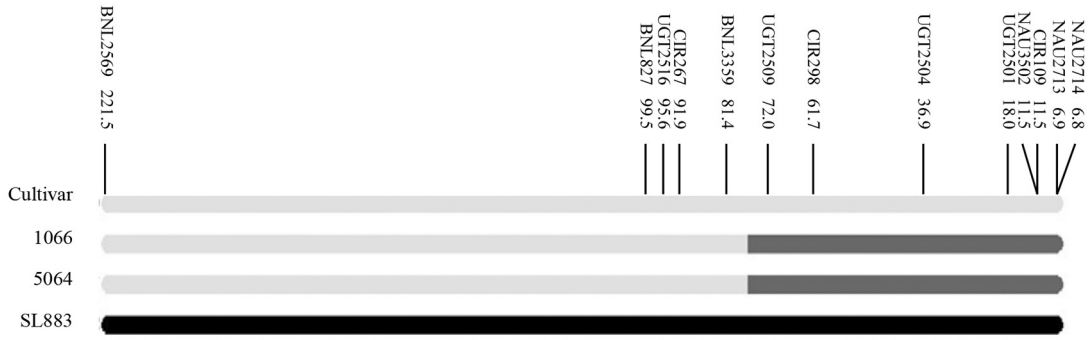

Fig. 1. Graphical genotype indicating physical positions of the simple sequence repeat (SSR) markers used to distinguish Sealand 883 (SL883) (black) and cultivar parent backgrounds (light gray). Recombinant individuals 1066 and 5064 are shown indicating their introgressed segment in a heterozygous state (dark gray). Marker distances are shown as physical distance in 10-kb units.

plots. Individual plants in 2014 and replicated progeny row plots in 2015 were ginned on laboratory gins, and fiber was sent to Cotton Incorporated for HVI testing. Measured HVI fiber parameters included those mentioned previously, although FL will be the focus of this discussion. Statistical analyses were conducted as described above.

RESULTS AND DISCUSSION

Bulked Sister Line Evaluation

The QTL(+) and QTL(−) lines used for this experiment were selected based on marker genotypes of the SSR loci flanking the *qFL-Chr.25* QTL. Prior research with this QTL showed a significant effect on FL (Kumar et al., 2019). However, significant differences existed for other fiber traits as well (Table 1), presumably due to SL883, which has many excellent fiber qualities. The main effect of year had a significant influence on average MIC, FL, STR, and SFC. The cultivar background main effect was significant for MIC, FL, UI, STR, ELO, and LP. This result was expected, as the cultivar parents selected for this study were purposely chosen for their phenotypic diversity and regional adaptation. The interaction of cultivar background with year was significant for MIC and SFC. The effect of the QTL [i.e., QTL(+) and QTL(−) lines], the cultivar genetic background parents (Acala SJ4, DP 50, GA089, and PM HS26), and the SL883 parent exhibited significant differences for MIC, FL, UI, STR, ELO, SFC, and LP. A QTL × genetic background interaction was significant for FL and LP. It is a common observation for a QTL to be influenced by the genetic background in which it is deployed, especially for quantitatively inherited traits such as FL and LP. These interactions only accounted for 8.9 and 18.7% of the total genetic sources of variance for FL and LP (Table 1), respectively, compared with the QTL source of variance, which accounted for 85.6 and 74.8% of the totals, respectively. A significant QTL × year effect was observed for SFC. No significant QTL × background × year effects were observed.

The parents used for this study were selected to represent the four major cotton growing regions in the United States, uniqueness of their fiber traits, and agronomic characteristics that make them desirable for those regions. The fiber quality diversity of these cultivars is presented in Table 2. The interspecific introgression parent, SL883, averaged an FL of 33.5 mm over the 2 yr that these lines

Table 1. ANOVA mean squares for highly related bulked sister lines harboring *qFL-Chr.25* deployed within four regionally adapted cultivar genetic backgrounds in Tifton, GA, in 2014 and 2015.

		Mean squares†						
Source	df	MIC	FL	UI	STR	ELO	SFC	LP
Year	1	326.3*	91.7*	507.4	963.4*	21,739.1	134.7*	6.4
Replication	1	2.8	0.6	0.2	28.2	0.1	1.9	0.5
Error A	1	0.1	0.5	30.1	5.3	1,719.5	0.2	0.3
Background	3	22.7**	23.4**	50.7*	46.7*	61,154.6**	6.7	28.3**
Background × year	3	35.4**	0.7	2.2	14.8	1,152.2	9.9*	0.3
Error B	10	3.2	0.5	10.8	7.7	1,084.1	2.4	0.2
QTL	4	270.8**	39.3**	28.7*	199.0**	63,783.4**	14.5**	8.0**
QTL × background	3	10.1	4.1*	19.5	8.1	461.6	5.5	2.0*
QTL × year	4	5.2	1.1	5.7	23.3	3,046.7	8.8*	0.1
QTL × background × year	3	3.4	0.2	5.3	11.6	3,104.5	2.3	0.1
Error C	274‡	8.8	1.2	11.4	19.4	2,685.2	2.9	0.5

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† MIC, micronaire; FL, upper half mean length; UI, uniformity index; STR, fiber bundle strength; ELO, elongation; SFC, short fiber content; LP, lint percentage.‡ Error C df for LPCT are 275.

were tested. The regionally adapted cultivar parents all had significantly shorter fiber than SL883, ranging from a low of 28.6 mm for DP 50 to a high of 29.9 mm for GA089. The QTL(−) BSLs averaged 31.5 mm, significantly lower than SL833, but significantly higher than all four regionally adapted parents. The FL of the QTL(+) lines were numerically higher than the QTL(−) lines by 0.9 mm; however, the difference was not statistically significant. This result indicates a large influence of the SL883 background on FL among the BSLs, as expected from a single-cross hybrid. The numerically higher FL for the QTL(+) lines supports the idea that the QTL influences FL over the cultivar background alone, although it appears there was recombination between the markers and trait, or too much environmental variance or experimental error to definitively link it to the QTL. The equation below was used to estimate number of replications that would have been required for the observed difference to have been significant:

$$\text{No. of replications} = 2\left(Z_{\alpha/2} + Z_{\beta}\right)\left(\sigma/\delta\right)^2$$

where $Z_{\alpha/2}$ is Z value at the acceptable Type I error rate; Z_{β} is Z value at the acceptable Type II error rate, σ represents the experimental standard deviation, and δ is the desired difference to be determined as significant. Based on the variance observed for FL in this experiment (1.08), it would have taken up to nine replications to detect a difference of 0.9 mm at a Type II error rate of 0.2 and Type I error of 0.05. Further testing across more environments and with more replication would likely have given more power to detect the positive influence of the QTL.

Micronaire for the four regional cultivars (Acala SJ4, DP 50, GA089, and PM HS26) ranged from 4.8 to 4.1, all of which are within the nondiscount range for MIC, which is 3.5 to 4.9 (USDA-FAS, 2018). Sealand 883 had a finer or more immature fiber with an average MIC value of 3.1, significantly lower than any of the groups in this study. Due to the relatively high STR, it is likely that the low MIC readings are due to fiber fineness rather than fiber immaturity. It is also interesting to note that the influence of SL883 on the lines with and without the *qFL-Chr.25* QTL was significant, both classes having low MIC values. It is to be expected that progeny derived from a single cross hybridization of the four regionally adapted cultivars with SL883 would have significant influence from SL883 on their fiber phenotypes. This is evident in the BSLs, which exhibit MIC values that are intermediate to the regionally adapted parents. The QTL(+) lines have significantly lower MIC values than the regionally adapted cultivars, though higher than SL883. The QTL(−) BSLs exhibit MIC values that are similar to the QTL(+) lines but are not significantly different than Acala SJ4 or PM HS26.

Uniformity index varied little among the BSLs (Table 2). Sealand 883 had the lowest UI of 83.3%, and PM HS26 had the highest with 85.4%. The other entries, including the BSLs, were intermediate, ranging from 84.8 to 84.1%. Fiber bundle strength was lowest for DP 50 with 270.9 kN m kg^{-1}. Sealand 883 had STR of 310.3 kN m kg^{-1}, which was not different from PM HS26 (312.6 kN m kg^{-1}) and GA089 (313.8 kN m kg^{-1}), respectively. The BSLs had similar STR to each other, SL883, Acala SJ4, GA089, and PM HS26 but were improved over DP 50. Elongation was highest for PM HS26 at 7.1% and lowest for SL883 with 4.4%. Elongation of the BSLs was intermediate to that of SL883 and PM HS26, and statistically not different from that of GA089, with 5.6% for QTL(−) lines, 5.3% for the QTL(+) lines, and 5.4% for GA089. Deltapine 50 exhibited the highest SFC of 7.9% compared with the rest of the lines, which ranged from 7.3 to 6.8%. Georgia 2004089 had the highest LP of 40.9%, and SL883 had the lowest LP of 27.8%. The QTL(−) and QTL(+) BSLs were not significantly different from each other in LP, with LP values of 32.9 and 32.3%, respectively.

Table 2. High volume instrument (HVI) fiber property means of highly related bulked sister lines differing for *qFL-Chr.25*, deployed within 4 regionally adapted genetic backgrounds compared to the cultivar parents and Sealand 883 (SL883) parent in Tifton, GA, in 2014 and 2015 field trials.

	HVI fiber property†						
Genotype‡	FL	MIC	UI	STR	ELO	SFC	LP
	mm	unit	%	kN m kg^{-1}	%		
QTL(–)	31.5b§	4.0cd	84.4ab	303.6ab	5.6c	7.3b	32.9c
QTL(+)	32.4b	3.7d	84.2bc	306.5ab	5.3c	7.1b	32.3cd
Acala SJ4	29.2cd	4.1bc	84.6ab	297.6b	6.2b	7.0b	32.7c
DP 50	28.6d	4.8a	84.1bc	270.9c	6.6b	7.9a	37.0b
GA089	29.9c	4.3b	84.8ab	313.8a	5.4c	7.3b	40.9a
PM HS26	29.0cd	4.2bc	85.4a	312.6a	7.1a	6.8b	30.3d
SL883	33.5a	3.1e	83.3c	310.3ab	4.4d	6.9b	27.8e
CV (%)	3.4	7.7	1.3	4.5	9.5	7.8	7.0

† FL, Upper half mean length; MIC, micronaire; UI, uniformity index; STR, fiber bundle strength; ELO, elongation; SFC, short fiber content; LP, lint percentage.

‡ QTL(+), lines with *qFL-Chr.25* from SL883; QTL(–), lines without *qFL-Chr.25* from SL883; DP 50, Deltapine 50; GA089, Georgia 2004089; PM HS26, Paymaster HS26.

§ Values within columns followed by a common letter are not different at k = 100 according to Waller–Duncan LSD.

The effect of *qFL-Chr.25* was limited when averaged across cultivar backgrounds, and although not statistically significant, 0.9 mm was a meaningful increase in fiber length averaged over 2 yr (Table 2). When analyzed separately by cultivar backgrounds, the effect of the QTL on FL becomes more evident (Supplemental Table S2). In addition to QTL, year also had a significant effect on FL test average for three of the four cultivar backgrounds. Significant differences in FL existed among the BSLs and parent cultivars for each cultivar background (Fig. 2).

Among the DP 50 BSLs, *qFL-Chr.25* provided a significant increase of 1.4 mm in FL for the QTL(+) lines over the QTL(−) lines (Fig. 2). Deltapine 50 exhibited the lowest FL in this test of 28.6 mm, compared with 33.5 mm for the SL883 parent. With no obvious *G. barbadense* in the pedigree (Kumar et al., 2019), DP 50 exhibited a noticeable increase in FL due to the presence of the SL883 background, as well as a large additional increase from the presence of the *qFL-Chr.25* QTL. The DP 50 BSLs averaged 32.7 mm in FL for the QTL(+) lines, and 31.3 mm for the QTL(−) lines, an improvement of 4.5% over the SL883 background alone.

Georgia 2004089, which had the longest FL (29.9 mm) of the upland cotton cultivar backgrounds used in this study, was derived from a cross of PD 94042/AP 7126, neither of which have any known *G. barbadense* introgression (Fig. 2). The effect of the SL883 background in the QTL(−) lines was a significant improvement of 1.9 mm over the GA089 parent. The effect of *qFL-Chr.25* in addition to the SL883 background was also significant, accounting for an additional 1 mm in FL over the QTL(−) lines, or a 3.1% increase due to the SL883 *qFL-Chr.25* allele alone.

In the Acala SJ4 genetic background, the *qFL-Chr.25* allele had no significant impact on FL over the baseline effect of the SL883 background genome alone (Fig. 2). These BSL groups were very similar in FL, 31.9 vs. 31.8 mm for the QTL(−) and QTL(+) BSLs, respectively. It is conceivable that Acala SJ4 might already harbor the

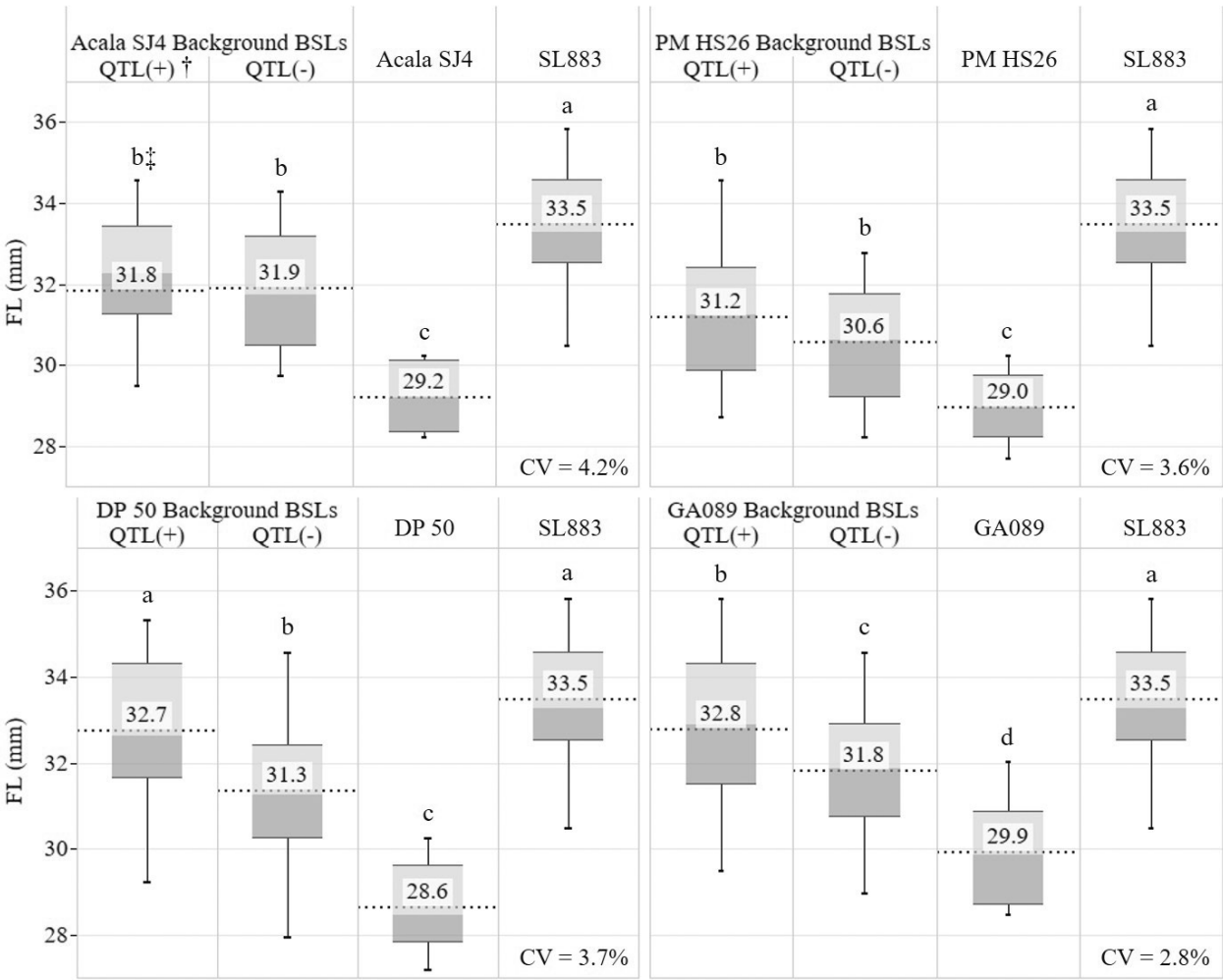

Fig. 2. Effect of the *qFL-Chr.25* quantitative trait locus (QTL) on upper half mean length (FL) deployed in highly related bulked sister lines (BSLs) within four regionally adapted cultivar genetic backgrounds at Tifton, GA in 2014 and 2015. † BSL, bulked sister line; QTL(+), lines with *qFL-Chr.25* from Sealand 883 (SL883); QTL(–), lines without *qFL-Chr.25* from SL883; PM HS26, Paymaster HS26; DP 50, Deltapine 50; GA089, Georgia 2004089. ‡ Values within cultivar background comparison windows followed by a common letter are not different at $k = 100$ according to Waller–Duncan LSD.

causal allele responsible for improved FL in this QTL region. Alternatively, the allele could have an undetectable effect due to epistatic interactions, or it could be masked by other beneficial fiber alleles Acala SJ4 already possesses in the QTL region. This would explain why the presence or absence of the region from SL883 has no effect on FL in addition to the effect of the SL883 background alone.

Similarly, FL of the PM HS26 BSLs harboring *qFL-Chr.25* did not differ significantly from their sister line counterparts, although there was a numerical mean difference of 0.6 mm (2%) between the QTL(+) and QTL(−) lines (Fig. 2). Paymaster HS26 is the result of a cross between Acala SJ4 and 5B9-184, a reselection from PM266 (Calhoun et al., 1997). The Acala cottons are thought to also have introgression from *G. barbadense* in their pedigrees (Smith et al., 1999) that have potentially conferred excellent fiber quality and resistance to diseases such as Verticillium wilt (caused by *Verticillium dahliae* Kleb.) (Zhang et al., 2005). Based on the pedigrees of these two cultivars, it seems plausible that Acala SJ4 could already harbor the allele responsible for improved FL, likely coming from *G. barbadense* for both SL883 and Acala SJ4. This result is quite interesting and suggests an avenue for further study.

The limited QTL effect in the Acala SJ4 and PM HS26 background BSLs could also have been caused by genotype × environment interaction. These genotypes were not tested in their region of adaptation, and therefore the effect of the QTL could have been masked by their unadapted phenotypic response to the growing environment in the lower southeastern United States, characterized by significant rainfall and humidity. In contrast, GA089 and DP 50 lines are in their preferred growing region. It is possible that testing the Acala SJ4 and PM HS26 derived lines in the southwestern United States (Maricopa, AZ) and the Texas High Plains (Lubbock, TX) would result in better expression of the QTL.

It was not surprising that BSLs, including the QTL(−) lines, would have FL longer than their cultivar parent genetic background due to segregation of other SL883 alleles. In each of the four genetic backgrounds, QTL(−) as well as the QTL(+) lines exhibited significantly longer FL than their cultivar parents (Fig. 2). Lack of significant *qFL-Chr.25* response is likely a function of limited replication rather than evidence disproving the research hypothesis. In all cases, the QTL(+) lines had improved FL compared to the QTL(−) lines. The low CV (3.4%) indicates the measured phenotypes are of high quality to determine efficacy of the QTL. This knowledge will be beneficial for designing and conducting future QTL assessment experiments.

Genotype × Environment Interaction of *qFL-Chr.25*

Analysis of the experimental variance revealed significant differences due to the Location effect for FL (Table 3). This indicates the testing environments should have been good for the differential expression of the QTL based on the background genotype's area of adaptation. The main effect of cultivar parent genetic background had a significant effect on FL. This is expected since the cultivars selected for this study vary considerably in fiber qualities. The QTL effect can be compared via four possible genotypic categories in this study: (i) the QTL allele from SL883 present in a cultivar background genome, (ii) without the QTL allele but still a hybrid progeny of a regionally adapted cultivar parent with SL883, (iii) original cultivar parent, or (iv) the SL883 parent. Among these categories, there were significant differences for FL.

The QTL × location interaction was significant for FL (Table 3); however, no significant differences existed due to the presence or absence of the SL883 allele at *qFL-Chr.25* (Fig. 3). Differences were due to changes in magnitude of the four genotypic classes rather than rank. Interestingly, the QTL × background and QTL × background × location interaction effects were not significant for FL. It was expected that genotypes with regionally adapted backgrounds would express the QTL disproportionately in their region of adaptation, and this was the primary reason for testing the QTL in these environments. However, more data were necessary to have allowed us to tease apart this relationship further.

Analysis of variance indicated that FL differences were significant for the cultivar background family groups with QTL(+) and QTL(−) lines combined (Table 3). The longest fibers, as measured by FL, were produced by SL883 with 33.2 mm, approaching the 35-mm length of pima cotton, or extra-long staple cotton. Upper half mean length among the cultivar backgrounds was highest for

Table 3. ANOVA mean squares for upper half mean length (FL) in field trials of *qFL-Chr.25* deployed within four regionally adapted cultivars compared with Sealand 883 in Maricopa, AZ; Lubbock, TX; Baton Rouge, LA; and Florence, SC in 2011.

Source	df	FL
Replication	1	0.024
Location	3	0.089*
Error A	3	0.005
Background	3	0.059**
Background × location	9	0.009
Error B	15	0.007
QTL	2	0.078**
QTL × location	6	0.006*
QTL× background	6	0.004
QTL× background× location	14	0.003
Error C	1520	0.002

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

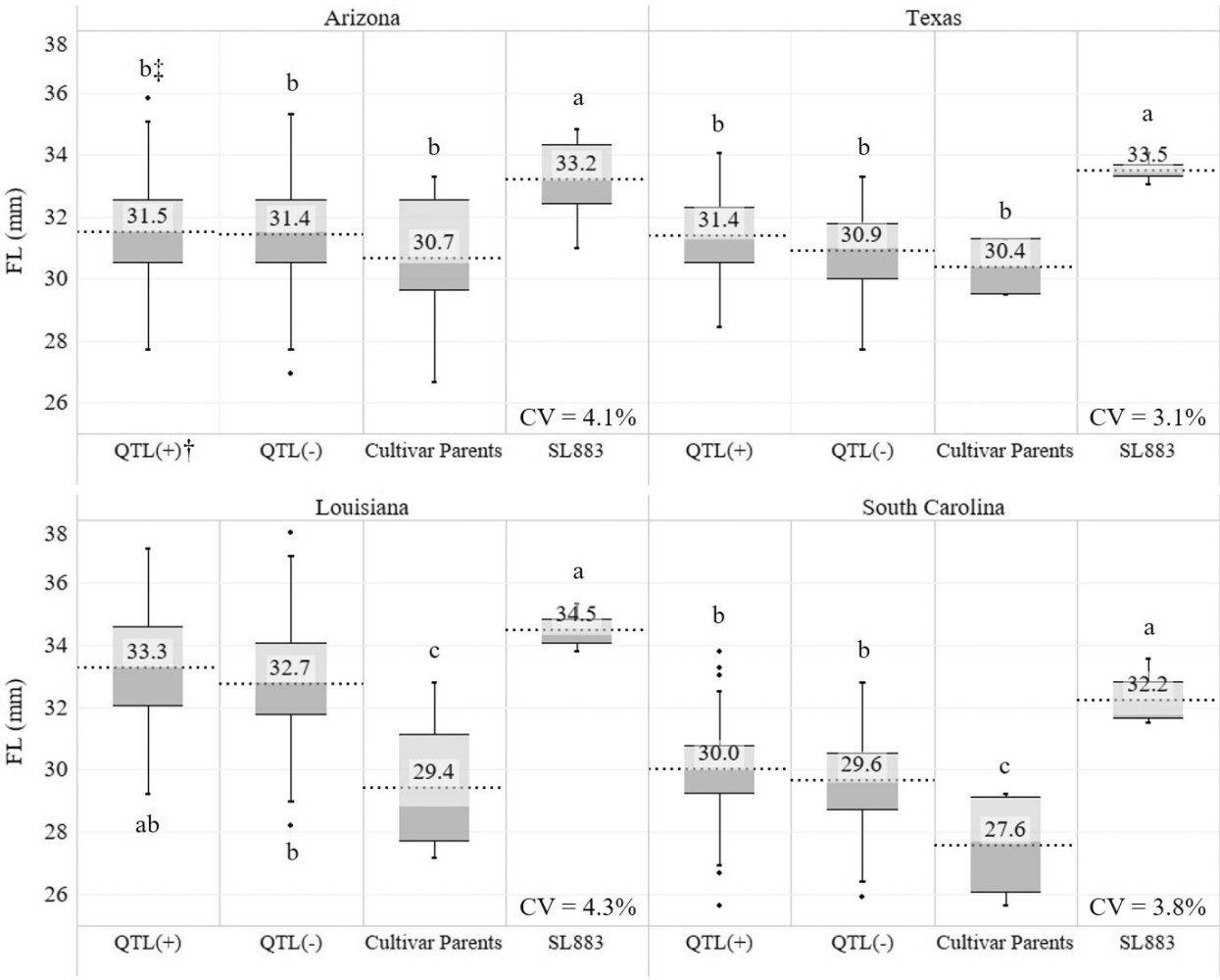

Fig. 3. Effect of *qFL-Chr.25* by location on upper half mean length (FL) encountered during field trials at Maricopa, AZ; Lubbock, TX; Baton Rouge, LA; and Florence, SC, in 2011. † QTL(+), lines with *qFL-Chr.25* from Sealand 883 (SL883); QTL(–), lines without *qFL-Chr.25* from SL883; PM HS26, Paymaster HS26; DP 50, Deltapine 50; GA089, Georgia 2004089. ‡ Values within cultivar background comparison windows followed by a common letter are not different at $k = 100$ according to Waller–Duncan LSD.

GA089, with 32.1 mm. The Acala SJ4 background lines had significantly shorter FL at 31.7 mm, followed by the DP 50 background with 31.0 mm, and PM HS26 was shortest with FL of 30.4 mm.

The QTL effect was significant, with QTL(+) lines grouped across backgrounds, QTL(−) lines across backgrounds, SL883, and cultivars combined across background. The FL values of the QTL(+) and QTL(−) lines were intermediate to those of SL883 and the regionally adapted cultivars. Sealand 883 exhibited FL of 33.2 mm across the testing sites, whereas the cultivar parents averaged far shorter FL of 29.6 mm. The hybrid progeny of the cultivar parents and SL883, both QTL(+) and QTL(−), were significantly shorter than SL883 but longer than their cultivar parents on average. The QTL(+) lines were not different statistically from those QTL(−) lines; however the FL mean was numerically higher for QTL(+) lines at 31.5 mm than for the QTL(−) lines with an average FL of 31.1 mm. We could not discern a significant increase overall based on the presence of the *qFL-Chr.25* QTL in addition to that of the SL883 genome alone. This is likely due to the small effect of the QTL, and/or the lack of sufficiently high numbers of replications within and/or across environments. It is also possible, if not likely, that recombination between *qFL-Chr.25* and the markers used to track it experienced recombination. In refining the QTL interval, CIR267 and BNL827 were found to recombine away from the QTL, and new SSR markers were identified with tighter linkage to *qFL-Chr.25*.

Refining the *qFL-Chr.25* Interval

To further refine the region containing *qFL-Chr.25*, recombinant individuals with crossovers in the previously defined QTL region were sought out. Two recombinant genotypes were identified after genotyping 1185 F_4 plants derived from heterozygous F_3s, Plant 1066 from the PM HS26 background, and Plant 5064 from the GA089 background. Four heterozygous F_7 individuals were selected from the PM HS26 background in 2013 (1066-73, 1066-77, 1066-78, 1066-93),

and three heterozygous F_7 plants from the GA089 background (5064-418, 5064-422, and 5064-440). These were selected to serve as segregating material to test the effect of this reduced *qFL-Chr.25* region in 2014 and 2015. Unfortunately, Genotypes 1066 and 5064 experienced crossovers in a similar region that eliminated the same ~60% of the introgressed SL883 chromosome segment. Figure 1 illustrates both families with cultivar-derived alleles at BNL827, UGT2516, and CIR267; and SL883 alleles for UGT2501, UGT2504, CIR298, and UGT2509.

The recombinant families only retained ~40% (814 kb) of the previously delineated 2.15-Mb region containing *qFL-Chr.25*. However, segregation of this smaller recombined segment had a significant effect on FL of the IPs tested in 2014 with at least $p \leq 0.10$ (Supplemental Table S3), indicating that this region harbors *qFL-Chr.25*. This was also evident in the IP-derived progeny rows with $p \leq 0.05$, tested in a replicated trial in 2015 (Supplemental Table S4). The families tested only as IPs in 2014, Families 1066-73 and 1066-77, from the PM HS26 background showed significantly longer FL when the SL883 portion of the recombined region was present, vs. the cultivar portion (Table 4). The effect on FL was an increase of 0.9, 1.5, and 1.3 mm for families 1066-73, 1066-77, and 1066-93, respectively, although 1066-93 was not significantly different. For the family derived from the GA089 background, 5064-418, the effect was an increase of 1 mm, significant at $\alpha = 0.10$. The CV was quite low for these families within each of the genetic classes, ranging from 3.2 to 4.1%.

Similar results were seen in the replicated testing of the segregating lines, where the effect of segregation of the ~800-kb SL883 region was statistically significant (Supplemental Table S4). For Family 1066-78, which is a PM HS26 background genotype, FL differed significantly for sister lines varying for the SL883 segment by 1.4 mm (Table 5). For the two GA089 families, 5064-422 and 5064-440, the effect of the SL883 portion of the chromosome segment improved FL significantly by 0.9 and 0.5 mm, respectively. Again, CVs were quite low, ranging from 2.9 to 3.2%. These small but significant effects are consistent with the previous performance of the QTL in highly related BSLs.

Although the two recombinant lineages, 1066 from the PM HS26 background and 5064 from the GA089 background, are unable to unequivocally resolve whether the region from SL883 harbors other QTL that influence FL, the evidence suggests that the area between CIR267 and BNL827 does not harbor *qFL-Chr.25*, and that the gene(s) is most likely within the region bound by UGT2509 and NAU2714 (Fig. 1). Additionally, the markers UGT2501, 2504, and 2509 are tightly linked to *qFL-Chr.25* and will be useful as selection tools to be applied in breeding programs for fiber length improvement using this allele from SL883.

CONCLUSION

When measured in genetically similar BSLs, the effect of the SL883 allele at *qFL-Chr.25* on FL was significant in two of the four cultivar backgrounds tested. The effect of the QTL, when averaged across the backgrounds tested in this trial, accounted for an additional 0.9 mm in the QTL(+) lines, though this was not significantly different from the QTL(−) lines. When separated by background, the effect of the QTL was not significantly greater than the SL883 background alone when deployed within Acala SJ4 or PM HS26. However, it was significant for the DP 50 and GA089 backgrounds, with FL increases of 1.4 (4.5%) and 1 mm (3.1%), respectively. The PM HS26 QTL(+) lines did have a numerically higher average FL that was 0.6 mm longer than the QTL(−) lines, though the difference was not statistically significant. To give these numbers perspective, in over 40 yr of breeding in the United States, the cotton crop has improved from an average FL of 26.7 mm in 1975 to 28.8 mm in 2016, an increase of only 2.1 mm (7.9%) (Cotton Incorporated, 2018).

Field trials planted across the Cotton Belt in 2011 did not show a significant effect due to the SL883 allele at *qFL-Chr.25*. This was likely due to either insufficient

Table 4. Upper half mean length means from individual plants of the three genetic classes of recombinant families for the region containing *qFL-Chr.25*, grown in Tifton, GA, in 2014.

	PM HS-26†						GA089‡	
***qFL-Chr.25* allele**	***n***	**1066-73§**	***n***	**1066-77§**	***n***	**1066-93§**	***n***	**5064-418§**
		mm		mm		mm		mm
Cultivar¶	14	29.8b#	16	28.6b	8	28.4a	29	30.7a
Heterozygous	28	29.6b	27	29.6a	25	29.1a	28	31.2a
Sealand 883	18	30.7a	12	30.1a	10	29.7a	15	31.7a
CV (%)		3.2		3.5		4.0		4.1

† PM HS-26, Paymaster HS26 background families;

‡ GA089, Georgia 089 background families

§ Families derived from individual heterozygous F_6 plants designated 1066-73, 1066-77, 1066-93, and 5064-418.

¶ Identity of simple sequence repeat (SSR) marker loci UGT2501 to UGT2509.

Values within columns followed by a common letter are not different at $k = 100$ according to Waller–Duncan LSD.

Table 5. Upper half mean length means from individual plants in 2014 and replicated plots in 2015 from three genetic classes of recombinant families for the region containing *qFL-Chr.25*, grown in Tifton, GA, in 2014 and 2015.

	PM HS26†		GA089‡			
qFL-Chr.25 allele	*n*	1066-78§	*n*	5064-422§	*n*	5064-440§
		mm		mm		mm
Cultivar¶	14	28.8c#	13	31.1c	23	31.1b
Heterozygous	30	29.4b	30	31.7b	33	31.4ab
Sealand 883	13	30.2a	17	32.0a	15	31.6a
CV (%)		3.2		2.9		3.2

† PM HS-26, Paymaster HS26 background families;

‡ GA089, Georgia 089 background families

§ Families derived from individual heterozygous F_6 plants designated 1066-78, 5064-422, and 5064-440.

¶ Identity of simple sequence repeat (SSR) marker loci UGT2501 to UGT2509.

Values within columns followed by a common letter are not different at $k = 100$ according to Waller–Duncan LSD.

replication, or due to recombination between the original markers used to track the QTL when advancing the lines for the genotype × environment experiments. Identification and testing of recombinant lines for the purpose of further refining the QTL interval suggested that *qFL-Chr.25* is tightly linked to newly developed markers UGT2501, UGT2504, and UGT2509. This region had a highly significant effect on lines from the PM HS26 and the GA089 backgrounds. Because testing to narrow down the region of interest was conducted on highly inbred recombinant sister lines, the data derived from the fiber samples were highly uniform and likely made the effect of the QTL that much more apparent.

Because QTL(−) lines exhibited FL significantly longer than the cultivar parents in each of the trials, it is likely that segregation of other SL883 alleles contributing to FL complicated the evaluation of the effect of *qFL-Chr.25*. However, the inability to show a statistically significant effect in some cases is likely due to limited biological replication rather than limited effect of the QTL. In the original mapping population, the segregation of *qFL-Chr.25* accounted for additive effects of 0.3 to 0.9 mm (Kumar et al., 2019), similar to the results seen here. Examining the effect of this QTL in a more highly isogenic state and with more biological replicates would be ideal and would be interesting for further study.

qFL-Chr.25 represents a *G. barbadense*-sourced QTL for the improvement of upland cotton germplasm. Along with the sequences for the tightly linked SSR markers (UGT2501, 2504, and 2509) included here, *qFL-Chr.25* from SL883 should prove to be beneficial in improving upland cotton germplasm. The portability of this QTL across diverse genetic backgrounds should make it valuable to breeders interested in improving fiber quality, a driving theme in cotton breeding today. Further, the utilization of foreign alleles from closely related species by way of introgression lines, such as the SL883 *qFL-Chr.25* allele, has the potential to make significant impacts on the cotton industry in relatively few years if deployed in commercial cultivars.

Supplemental Material

Supplemental material is available online for this article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Standards

The experiments described herein comply with current laws of the country in which they were performed.

Acknowledgments

We thank Jennifer McBlanchett for technical assistance and the Cotton Incorporated Fiber Testing Laboratory for providing HVI fiber quality data. We gratefully acknowledge the partial funding of this research by the National Science Foundation Partnerships for Innovation (IIP-091856), USDA Initiative for Future Agriculture and Food Systems (0052100-9685), Georgia Cotton Commission, and Cotton Incorporated for funding N. Brown's Ph.D. research and education through the Cotton Incorporated Fellowship Program.

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