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RESEARCH

Utility Assessment of Published Microsatellite Markers for Fiber Length and Bundle Strength QTL in a Cotton Breeding Program

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ABSTRACT

Numerous DNA markers associated with quantitative trait loci (QTL) for cotton (*Gossypium* spp.) fiber quality traits have been identified in the literature, but there are still significant challenges regarding the use of these QTL in marker-assisted selection. While one of the primary limitations to the application of marker-assisted selection for fiber quality traits has been the inconsistency of marker–trait associations, more recent studies have reported numerous marker–trait associations and colocating of QTL in different genetic backgrounds and environments. The objectives of this study were to assess the published microsatellite markers linked to upper-half mean fiber length (UHML) and fiber bundle strength (Str) QTL in different genetic backgrounds and to characterize the utility of stable marker–trait associations in selection for improved fiber quality within the context of an applied breeding program. Using the results of 32 published QTL mapping studies, six stable marker–trait associations each for UHML and Str were detected. For each trait, the mean of $F_{3:4}$ progeny rows that were grouped based on the six marker genotypes was compared with the mean of $F_{3:4}$ progeny rows that were grouped based on phenotype. In all but one case, the mean UHML and Str of $F_{3:4}$ progeny rows with the majority of alleles (i.e., four to six alleles) in the desirable state was similar to the mean of $F_{3:4}$ progeny rows derived from F_3 plants in the top 20% for UHML and Str. Our results indicate that, after proper validation, published QTL for UHML and Str could be utilized in selection for improved fiber quality.

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Abbreviations: HVI, high-volume instrument; MAS, marker-assisted selection; QTL, quantitative trait locus (loci); RIL, recombinant inbred line; SSR, microsatellite marker; Str, fiber bundle strength; UHML, upper-half mean fiber length.

Cotton (*Gossypium* spp.) is the most widely planted row crop in Texas, providing approximately $2 billion in gross value of production to the state (USDA, 2015b). The US reliance on export markets, coupled with excess global supply, dictates that Texas cotton production must compete globally, not only in terms of lint yield but also fiber quality (Meyer et al., 2015; USDA, 2015a). Consequently, growing global demand for longer, stronger, and more uniform fibers has led to an increased emphasis on breeding for improved cotton fiber quality (Smith et al., 2009; Cantrell et al., 2000; Meredith and Nokes, 2011; Bourland and Jones, 2012). There are four species of cultivated cotton, but upland cotton (*Gossypium hirsutum* L.) cultivars account for the vast majority of global cotton production, owing to high yield and broad adaptation. Therefore, the majority of cotton breeding programs in the United States focus primarily on genetic improvement within the upland cotton gene pool.

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Improvement of fiber quality traits through conventional breeding within upland cotton germplasms has been somewhat impeded by the negative relationship between lint yield and fiber quality traits (Al-Jibouri et al., 1958; Miller et al., 1958; Meredith, 1984; McCall et al., 1986; Smith and Coyle, 1997; Hinze et al., 2011; Ulloa, 2006) and low genetic diversity (Van Esbroeck and Bowman, 1998; Lacape et al., 2007; Hinze et al., 2012; Fang et al., 2013). Molecular breeding methods, such as quantitative trait loci (QTL) mapping and marker-assisted selection (MAS), show potential to help mitigate some of these challenges (Chee and Campbell, 2009; Fang, 2015). More specifically, knowledge of specific QTL regulating the phenotypic expression of fiber length and strength may be used in MAS to more efficiently select for both improved lint yield and fiber quality. Numerous QTL mapping studies have been conducted over the past two decades to investigate the genetic basis of fiber quality traits in cotton and develop genetic markers for use in MAS. Collectively, these QTL analyses suggest that fiber length and strength are controlled by many genes of relatively minor effect, which is congruent with the findings of traditional quantitative genetic studies (Meredith 1984). Said et al. (2013) published a meta-analysis of QTL mapping studies, nearly half of which were conducted on fiber quality traits. Among the 20 QTL mapping publications for fiber quality reviewed, the majority were conducted in either upland intraspecific populations (8) or *Gossypium hirsutum* × *Gossypium. barbadense* L. populations (11). Fiber length and strength were the most comprehensively studied traits, and the meta-analysis integrated 151 and 132 reported QTL for fiber length and strength, respectively.

Despite the multitude of QTL for fiber quality traits described in the literature, there are much fewer reports of these QTL being used in MAS (Zhang et al., 2003; Guo et al., 2005; Shen et al., 2011). Breeding programs still face many challenges in the application of MAS for quantitative traits, such as fiber length and strength, with one of the primary limitations being the inconsistency of marker–trait associations across environments and genetic backgrounds (Fang, 2015). This inconsistency may be attributed to the use of different experimental populations and epistatic interactions, particularly in cotton, where numerous QTL mapping studies have been conducted in interspecific populations (*G. hirsutum* × *G. barbadense*) owing to low polymorphism among elite upland cotton germplasms and superior fiber quality present in *G. barbadense* (Shen et al., 2006; Fang, 2015). Inconsistency among marker–trait associations across mapping studies may also be attributable to QTL × environment interactions (Paterson et al., 2003; Lacape et al., 2010), the use of nonrobust genetic markers, such as random amplified polymorphic DNA (RAPD), or differences in experimental designs and statistical analyses (Fang, 2015).

More recent QTL mapping studies for fiber quality traits have used larger intraspecific upland populations, replicated experimental designs, multiple testing locations, and more complex mating designs to improve the accuracy and reliability of QTL estimates (Qin et al., 2008; Lacape et al., 2010; Zhang et al., 2013). Association mapping studies utilizing diverse sets of upland cotton germplasms have also been used to identify more stable QTL for fiber quality traits (Abdurakhmonov et al., 2008; Cai et al., 2014; Fang et al., 2014). As a result, the number of marker–trait associations and colocating fiber quality QTL reported across different genetic backgrounds and environments has increased (Shen et al., 2006; Cai et al., 2014; Fang et al., 2014). These genetic markers linked to stable fiber quality QTL are likely superior candidates for MAS. Still, supporting research is necessary to assess the breadth and utility of previously reported marker–trait associations, which will ultimately aid in the development of effective molecular-assisted breeding methods for fiber quality traits.

Thus, the objectives of this research were (i) to assess marker–trait associations of published microsatellite markers (SSRs) linked to upper-half mean fiber length (UHML) and fiber bundle strength (Str) QTL in three genetic backgrounds and (ii) to characterize the utility of stable marker–trait associations regarding selection for improved fiber quality within a conventional cotton breeding program.

MATERIALS AND METHODS

Plant Material

Three experimental populations in the breeding pipeline at the Texas A&M AgriLife Research (AgriLife) Cotton Improvement Lab were selected for the study based on pedigrees and fiber quality characteristics. Two of the populations were derived from crosses between four AgriLife upland cotton experimental lines, 04WL-19/09207 (population A), and 09 PP-03–02/09917 (population B)—all internal, unreleased breeding lines with improved fiber quality. The progenitor lines of 04WL-19 were 'Acala 1517–99' (Cantrell et al., 2000; PI 612326), TAM 96WD-18 (Thaxton et al., 2005; PI 635879), TAM 91C-95Ls (Smith, 2001; PI 614952), and TAM 94L-25 (Smith, 2003; PI 631440). The experimental line 09207 was derived from a cross between HAR U 585–12 (*Gossypium hirsutum* × *Gossypium arboretum* L. × *Gossypium raimondii* Ulb.) (PI 529381), an accession from Cote D'Ivoire, and TAM B182–33, an extra-long staple upland (ELSU) germplasm line (Smith et al., 2009; PI 654362). The experimental line 09 PP-03–02 was derived from a cross between 03 HIL B147–23 and 03 HIL B182–34, both unreleased AgriLife experimental lines with exceptional fiber length. The experimental line 09917 was derived from a cross between TAM 96WD-18 and 'Tamcot 73' (Smith et al., 2011; PI 662044). The third population (C) was selected as a comparison to populations A and B in regard to fiber quality. Population C was derived from a cross between TMC-9–2, an unreleased AgriLife experimental exotic introgression line

('Tamcot Pyramid' [Thaxton and El-Zik, 2004; PI 617042]/['TM-1'/*Gossypium mustelinum* Miers ex Watt]), and the upland cultivar LA 887 (Jones et al., 1991; PI 547084). The pedigrees of these three populations include diverse set of germplasm lines that are representative of the overall breeding program.

Field Study

Within the AgriLife Cotton Improvement Lab breeding program, pedigree selection is typically used after hybridization to select elite germplasm lines (Smith, 2003; Smith et al., 2009). Although UHML and Str are quantitatively inherited traits, selection is generally practiced in early generations due to moderate-to-high heritability and the predominantly additive nature of genetic variation for these traits (Al-Rawi and Kohel, 1970; Baker and Verhalen, 1973; May, 1999; Jenkins et al., 2009). Thus, the utility of previously reported marker–trait associations for UHML and Str was assessed in the context of the standard breeding program (i.e., early generation selection within a pedigree selection scheme). The F_2 seeds derived from each cross described above were planted as a single-row plot (13.1 × 1 m; approximately 10 plants m^{-1}) in 2012 at College Station, TX, and open pollinated seeds were bulk harvested from each row to derive three segregating F_3 populations (populations A, B, and C). Low levels of outcrossing (<2%) were expected, considering the minimal pollinator activity in the area (Simpson, 1954; Meredith and Bridge, 1973; Smith, unpublished data, 1989).

The three F_3 populations were grown at the AgriLife Research Farm near College Station, TX on a Weswood silt loam (fine-silty, mixed, thermic Fluventic Ustochrept) integrated with Ships clay (very fine, mixed, thermic Udic Chromustert). Standard cultural practices for cotton production in central Texas were conducted, including pesticide and herbicide applications and furrow irrigation. Nineteen rows (13.1 × 1 m) of each F_3 population were planted on 22 Apr. 2013, and plants within each row were thinned to a density of approximately one plant per 0.4 m. A total of 731 F_3 individual plants were selected randomly in 2013 and tagged in the field, specifically 269 plants within population A (04WL-19/0927), 243 plants within population B (09PP-03–02/09917), and 219 plants within population C (TMC-9–2/LA 887). Fifteen fully-developed open bolls were harvested from each tagged plant in late October 2013 and ginned on a 10-saw laboratory gin without lint cleaners. Fiber properties, including high-volume instrument (HVI)-measured UHML and Str, were determined at Texas Tech University's Fiber and Biopolymer Research Institute in Lubbock, TX.

Considering that selection for UHML and Str is generally practiced at the F_3 generation in the AgriLife Cotton Improvement Lab breeding program, approximately half of the harvested F_3 plants were selected randomly and planted as unreplicated $F_{3:4}$ progeny rows (1 × 9 m) on 6 May 2014. One hundred and thirty-one F_3 plants were selected within population A, 120 within population B, and 108 within population C. Boll samples (30 first- and second-position bolls from the middle of the fruiting zone) were hand harvested from each $F_{3:4}$ progeny row in late October 2014. Seed cotton samples were ginned on a laboratory saw-gin without lint cleaners, and fiber properties were determined at the Fiber and Biopolymer Research Institute in Lubbock, TX using the HVI system.

Genotyping

Young leaf tissue was collected during the summer of 2013 from each of the 731 tagged F_3 plants, and genomic DNA was extracted using a modified CTAB (cetytrimethylammonium bromide) method, described by Zhang et al. (2010). All genotyping was conducted at the USDA–ARS Cotton Fiber Bioscience Research Unit at the Southern Regional Research Center in New Orleans, LA. Seeds of the parental lines 04WL-19, 0927, 09 PP-03–02, 09917, and TMC-9–2 were not available. Thus, tissue could not be obtained for DNA extraction and polymorphism screening of five parental lines. Instead, DNA was extracted from the progenitors of each parental line as a representation of putative alleles. For example, the progenitor lines of 09207 were HAR U 585–12 and TAM B182–33 ELS. No germplasm was available to represent TMC-9–2. Ultimately, DNA samples from the progenitor lines TAM 94L-25, TAM 96WD-18, TAM 91C-95Ls, TAM B182–33, 03 HIL B147–23, 03 HIL B182–34, Tamcot 73, Acala 1517–99, HAR U 585–12, and LA 887 were screened with 516 SSR primer pairs selected from 32 published fiber quality QTL mapping studies (Table 1).

Primer sequences for the selected SSRs were obtained from CottonGen (https://www.cottongen.org). Two hundred and twenty-one of the SSRs were polymorphic between at least two of the progenitor lines and were used to genotype the F_3 plants. Multiplex polymerase chain reaction (PCR) was performed when genotyping the F_3 progeny. Forward primers were fluorescent labeled at the 5′ end with 6-FAM (6-carboxyfluorescein), HEX (4, 7, 2′, 4′, 5, 7-hexachloro-carboxyfluorescein, or NED (7′, 8′-benzo-5-fluoro 2′, 4, 7,-trichloro-5-carboxyfluorescein). Polymerase chain reaction conditions used for the SSR primer pairs were described by Fang et al. (2010). Amplified fragments with fluorescent labels were separated and sized by an automated capillary electrophoresis system ABI 3730XL (Applied Biosystems Inc., Foster City, CA) using GeneScan-400™ ROX® as the internal

Table 1. Publications from which the 516 microsatellite markers (SSRs) were selected.

Publication	Type of population†	Publication	Type of population
Cai et al. 2014	Intraspecific	Shen et al. 2005	Intraspecific
Chen et al. 2009	Intraspecific	Shen et al. 2006	Intraspecific
Fang et al. 2014	Intraspecific	Shen et al. 2007	Intraspecific
Frelichowski et al. 2006	Interspecific	Su et al. 2013	Interspecific
Gore et al. 2014	Interspecific	Tan et al. 2015	Intraspecific
He et al. 2007	Interspecific	Wang et al. 2006	Intraspecific
Islam et al. 2014	Intraspecific	Wang et al. 2013a	Interspecific
Kim et al. 2013	Intraspecific	Yu et al. 2013a	Interspecific
Lacape et al. 2005	Interspecific	Yu et al. 2013b	Interspecific
Lacape et al. 2010	Interspecific	Zeng et al. 2009	Interspecific
Li et al. 2013	Interspecific	Zhang et al. 2003	Intraspecific
Lin et al. 2005	Interspecific	Zhang et al. 2005	Intraspecific
Park et al. 2005	Interspecific	Zhang et al. 2009	Intraspecific
Qin et al. 2008	Intraspecific	Zhang et al. 2012	Intraspecific
Rong et al. 2004	Interspecific	Zhang et al. 2013	Intraspecific
Said et al. 2013	Inter- and Intraspecific	Zhiyuan et al. 2014	Intraspecific

† "Intraspecific" refers to populations derived from *G. hirsutum* cultivars/accessions, and "interspecific" refers to populations derived from hybridization between *G. hirsutum* and another tetraploid *Gossypium* species.

DNA standard. Allele calling was performed using GeneMapper 4.0 software (Applied Biosystems Inc., Foster City, CA).

Statistical Analysis

Frequently, multiple loci (i.e., more than two alleles) were amplified by a single SSR primer pair, resulting from the primers annealing either to homologous loci within the A and D subgenomes or to homeologous loci across subgenomes. A notable limitation of this study was the lack of the direct parental genotypes. As we were unable to construct genetic maps for the three populations without the parental genotypes, each SSR allele was treated as a dominant marker by scoring alleles as either 'present' or 'absent.' Thus, each SSR allele was treated as a separate locus in the statistical analysis. The genomic position of each SSR primer pair was estimated according to the linkage group(s) specified in CottonGen (https://www.cottongen.org). The majority of SSR primer pairs had been mapped to multiple chromosomes according to the CottonGen database, either within the same mapping population or across different mapping populations.

A two-step approach, described by Dudley (1993), combining analysis of variance (ANOVA) followed by stepwise multiple linear regression was used to identify SSR alleles associated with UHML and Str within and across the F_3 populations. More recent studies have used this approach to evaluate marker–trait associations for agronomic and quality traits in wheat (*Triticum aestivum* L.) (Jia et al., 2011) and disease-resistance loci in maize (*Zea mays* L.) (Chung et al., 2010). Statistical analyses were conducted using JMP® v12.0.1 (SAS Institute, 2013), and all models conformed to the assumptions of ordinary least squares estimation, including the normality and homogeneity of residuals. First, the two-step analysis was performed within each population, and the results were compared across genetic backgrounds to identify any common SSRs associated with UHML and Str. Microsatellite marker alleles having a score in ≤5% of either the present or absent categories (i.e., rare alleles) were excluded from the analyses within a specific population to satisfy the assumptions of ordinary least squares estimation. One-way ANOVA was performed to identify individual alleles associated with UHML and Str among the F_3 plants derived from each population. The effect of each allele was analyzed as a fixed effect, and a less stringent probability level of $\alpha = 0.05$ was used due to the likelihood of committing type II errors. Alleles having a significant association with UHML and Str among the F_3 plants, as identified by ANOVA, were selected. Next, stepwise multiple linear regression was conducted to analyze the joint effects of the selected alleles and to ultimately identify a core subset of alleles associated with UHML and Str within each F_3 population. Mixed (i.e., forward and backward) stepwise linear regression was conducted using a probability-to-enter and a probability-to-leave of $\alpha = 0.05$. The associations between SSR alleles and UHML and Str, identified through the two-step analysis, were also evaluated in the $F_{3:4}$ progeny rows grown in 2014 through multiple regression on the identified subset of alleles.

The two-step analysis described above was also conducted across populations using the combined data set to identify SSRs associated with UHML and Str. The analysis conducted across populations was similar to the analysis conducted within each population, except the following ANOVA model accounting for the effect of genetic background was used to identify alleles associated with UHML and Str across populations:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} represents the observed UHML or Str of all F_3 plants grown in 2013, μ represents the overall mean, α_i represents the main effect of the ith population, β_j represents the main effect of the jth allele, $\alpha\beta_{ij}$ represents the interaction effect of the ith population and jth allele, and ε_{ijk} represents the residual error. Alleles having a significant association with UHML and Str among the F_3 plants were selected. Interaction plots for selected alleles for which the population × allele interaction effect was significant were examined, and alleles associated with opposite effects on UHML or Str within different genetic backgrounds were excluded from further analyses. These steps were performed to select genetic markers that would enable selection for improved fiber quality across populations within a breeding program. Next, stepwise multiple linear regression was conducted to collectively analyze the effects of the selected alleles and to identify a core subset of alleles associated with UHML and Str among the F_3 plants across genetic backgrounds. The associations between SSR alleles and UHML and Str identified through the two-step analysis were also evaluated in the $F_{3:4}$ progeny rows through multiple regression on the identified subset of alleles.

In order for stable marker–trait associations to have widespread utility within a breeding program, they must also be effective in selection for fiber quality across populations. Thus, the utility of stable marker–trait associations for UHML and Str in the three populations was evaluated by comparing the mean UHML and Str of $F_{3:4}$ progeny rows with the majority of alleles in the desirable state with that of the $F_{3:4}$ progeny rows derived from F_3 plants in the top 20% for UHML and Str, as measured by HVI. The SSR alleles identified through the two-step analysis across populations were used to select F_3 plants for which the majority of alleles were in the beneficial state. All mean comparisons were conducted using Fisher's Protected Least Significant Difference (LSD), and Pearson product moment correlation coefficients were used to measure the phenotypic correlations between UHML and Str within and across the three populations.

RESULTS AND DISCUSSION

Populations A and B had superior fiber quality in comparison with population C (Table 2). A list of all 516 SSRs can be found in Supplemental Table 1 that indicates which SSRs were polymorphic among at least two of the progenitor/parental lines, along with the multiplex set and fluorescent label used for each primer pair used to genotype the F_3 progeny. Five hundred and fourteen alleles were amplified by the 221 polymorphic SSR primer pairs.

Marker–Trait Associations within Populations

There were similar levels of polymorphism among all three populations. Three hundred and seventy five of 514 SSR alleles were segregating (i.e., greater than 5% within 'presence' or 'absence' categories) within population A, 381 alleles were segregating within population B, and 365 alleles were segregating within population C.

Table 2. Summary statistics of upper-half mean length (UHML) and bundle strength (Str) among F_3 individual plants within three populations evaluated at College Station, TX, in 2013.

	Population A (*04 WL-19/09207*)	Population B (*09 PP-03–02/09917*)	Population C (*TMC-9–2/LA 887*)
N	269	243	219
UHML		mm	
Mean	33.5	32.7	28.5
SD	1.6	2.2	1.6
Maximum	38.4	37.8	33.5
Minimum	29.2	25.1	25.1
Median	33.5	32.8	28.4
Str		kN m kg^{-1}	
Mean	360.7	365.3	322.0
SD	21.4	21.3	24.0
Maximum	423.8	435.6	372.8
Minimum	306.1	298.2	271.7
Median	361.0	365.9	318.8

Upper-Half Mean Fiber Length

The population-specific regression models resulting from the two-step analysis retained 15, 9, and 14 alleles associated with UHML within populations A, B, and C, respectively (Table 3). Alleles amplified by the CIR246 and C2–114 primer pairs were strongly associated with UHML among the F_3 plants within the two populations with superior fiber quality (populations A and B), and alleles amplified by the BNL1604 primer pair were strongly associated with UHML among F_3 plants within populations A and C. Less than half of the SSR alleles maintained an association with UHML across both years. Only 2 of 15 alleles, $BNL1604_{113}$ and $C2–114_{149}$, were associated with UHML among the $F_{3:4}$ progeny rows within population A. Both SSRs had been mapped previously to chromosome 7 (Said et al., 2013; Fang et al., 2014); thus, it is possible that the two markers are linked to a single QTL for UHML. Within population B, 3 of 10 alleles maintained an association with UHML among the $F_{3:4}$ progeny rows. Among the three alleles, DPL0570 and NAU1043 had been previously mapped to chromosomes 11 and 7, respectively (Shen et al., 2005; Fang et al., 2014), while DPL1201 had not been mapped to a known linkage group (Fang et al., 2014). Interestingly, NAU1043 had previously been reported as linked to QTL for UHML in four separate studies (Shen et al., 2005; Wang et al., 2006; Yu et al., 2013a; Cai et al., 2014). Together, these results may suggest the presence of a stable QTL for UHML on chromosome 7. Only three alleles, $BNL4300_{175}$, $HAU0087_{190}$, and $NAU1167_{189}$, were associated with UHML across both years among the F_3 and $F_{3:4}$ progeny within population C.

When the analysis was performed separately within each population, we detected no SSR primer pairs associated with UHML across multiple populations and both years. The majority of SSR–UHML associations identified among the F_3 plants within each population were not statistically significant when regressed on the UHML of the $F_{3:4}$ progeny rows. The inability to detect these associations among the $F_{3:4}$ progeny rows may be attributable to several factors—first and foremost is sample size (Beavis, 1998). The population size of the $F_{3:4}$ progeny rows was approximately half of the sampled F_3 plants. Second, QTL × environment interactions may also account for the lack of association with $F_{3:4}$ progeny row UHML. Although traditional quantitative genetic studies have shown that genotype × environment interaction effects are generally minor regarding UHML(Al-Jibouri et al., 1958; Miller et al., 1958; Abou-El-Fittouh et al., 1969; Meredith and Bridge, 1973; Chee and Campbell, 2009; Lacape et al., 2010), several mapping studies have reported significant QTL × environment interactions, demonstrating that the expression of some QTL for UHML is dependent on environmental conditions during fiber development (Paterson et al., 2003; Shen et al., 2006; Sun et al., 2012). Third, recombination events between the SSR allele and QTL for UHML may have contributed to the loss of the statistical association.

Fiber Bundle Strength

The population-specific regression models resulting from the two-step analysis retained 11 alleles associated with Str within population A, 5 alleles within population B, and 16 alleles within population C (Table 4). Alleles amplified by the primer pair BNL1604 were associated with Str among the F_3 plants within all three populations. Additionally, alleles amplified by NAU2291 (same forward and reverse primers as NAU0913) were associated with Str among F_3 plants derived from populations B and C, and alleles amplified by BNL3031 were associated with Str among F_3 plants derived from populations A and C. Similar to the findings for UHML, less than half of the alleles maintained an association with Str among the $F_{3:4}$ progeny rows evaluated in 2014. Five of 11 alleles remained associated with Str within population A; one of five alleles remained associated with Str within population B; and 5 of 16 alleles remained associated with Str within population C. $BNL1604_{98}$ was associated with Str among progeny derived from populations A and B across both years.

Marker–Trait Associations across Populations

Over half (55.45%) of the alleles scored ≤5% in the 'presence' or 'absence' category within one or more of the populations. These alleles were excluded from the analysis across populations, resulting in a total of 229 shared polymorphic SSRs. Thus, it is important to note that the results presented below are specific to the genetic diversity present across all three populations.

Table 3. Multiple regression models of selected microsatellite marker (SSR) alleles associated with variation in upper-half mean length (UHML) within each population of F_3 individual plants and the resulting $F_{3:4}$ progeny rows when grown at College Station, TX, in 2013 and 2014, respectively.

Term†	F_3 estimate‡	$F_{3:4}$ estimate	Publication	Chr.§
Population A				
(*04 WL-19/09207*)				
Intercept	33.84***	32.12***		
$BNL0830_{104}$	–0.24**	–0.15	Said et al. 2013	15
$BNL1604_{113}$	**–0.23****	**–0.39****	Said et al. 2013	7, 16, 17
$BNL3408_{130}$	–0.17*	–0.07	Said et al. 2013; Wang et al. 2013a; Zeng et al. 2009	3, 17
$C2–114_{149}$	**–0.33*****	**–0.27***	Fang et al. 2014	7
$CGR6383_{223}$	0.38***	0.18	Fang et al. 2014; Islam et al. 2014	3, 14
$CIR246_{146}$	0.27***	0.07	Qin et al. 2008; Wang et al. 2006	14
$DPL0236_{154}$	–0.19*	0.10	Fang et al. 2014	N/A
$DPL1931_{324}$	–0.35*	–0.21	Gore et al. 2014	11
$JESPR065_{165}$	–0.35***	–0.21	Shen et al. 2006; Shen et al. 2007	5, 7, 21, 22, 26
$JESPR208_{108}$	–0.39***	–0.15	Shen et al. 2007	9, 23
$JESPR218_{104}$	0.46***	0.10	Cai et al. 2014	19
$NAU1035_{176}$	0.33***	0.14	Zhang et al. 2012	23
$NAU1190_{216}$	0.20**	0.13	Kim et al. 2013; Wang et al. 2013a	3
$NAU2508_{152}$	–0.28**	–0.10	Cai et al. 2014; Zhang et al. 2012	10
$NAU3700_{194}$	0.34***	0.17	Zhiyuan et al. 2014	17
Adjusted R^2	0.533	0.216		
Population B				
(*09 PP-03–02/09917*)				
Intercept	32.00***	31.08***		
$C2–114_{149}$	–0.31*	–0.18	Fang et al. 2014	7
$CIR167_{207}$	–0.29*	–0.31	Zeng et al. 2009	12, 26
$CIR246_{168}$	–0.69**	–0.16	Qin et al. 2008; Wang et al. 2006	14
$DPL0570_{302}$	**0.39****	**0.42****	Fang et al. 2014	11
$DPL1201_{275}$	**–0.48****	**–0.37***	Fang et al. 2014	N/A
$HAU0880_{172}$	–0.39**	0.01	Tan et al. 2015; Wang et al. 2013a	2, 17
$NAU2291_{206}$	–0.33*	–0.06	Fang et al. 2014; Said et al. 2013	4, 22
$NAU1043_{227}$	**0.50****	**1.09*****	Shen et al. 2005; Wang et al. 2006; Yu et al. 2013a; Cai et al. 2014	7
$SHIN-1635_{241}$	0.34*	0.05	Fang et al. 2014	14
Adjusted R^2	0.187	0.224		
Population C				
(*TMC-9–2/LA 887*)				
Intercept	29.63***	29.95***		
$BNL1231_{190}$	0.24**	–0.01	Said et al. 2013; Wang et al. 2006	11, 21
$BNL1604_{120}$	0.27**	0.21	Said et al. 2013	7, 16, 17
$BNL1667_{164}$	0.32***	0.12	Rong et al. 2004; Said et al. 2013	1, 2, 14, 15
$BNL3400_{175}$	**0.27****	**0.46****	Fang et al. 2014	5, 19
$BNL3594_{199}$	–0.26**	–0.10	Said et al. 2013	6, 25
$CIR212_{141}$	0.37***	0.06	Said et al. 2013	3, 19
$HAU0087_{190}$	**–0.33*****	**–0.30***	Fang et al. 2014	4, 22
$MUSS422_{200}$	–0.25**	–0.10	Said et al. 2013	15
$NAU1167_{189}$	**0.27****	**0.57*****	Cai et al. 2014	3, 17
$SHIN-0384_{185}$	–0.24**	–0.24	Fang et al. 2014	24
$TMB0338_{226}$	0.20*	0.22	Fang et al. 2014	25
$TMB1409_{192}$	–0.33*	–0.23	Fang et al. 2014	16
$TMB1409_{195}$	0.26*	0.20	Fang et al. 2014	16
$TMB1898_{223}$	0.20*	0.24	Kim et al. 2013	3
Adjusted R^2 =	0.562	0.441		

* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level; alleles in bold were significantly associated with UHML among the F_3 individual plants and $F_{3:4}$ progeny rows evaluated in 2013 and 2014, respectively.

† The 'present' allelic state was used as the base level for regression estimates for each allele. Positive or negative numbers indicate the direction of the associated response.

‡ The estimates from 2013 F_3 individual plant UHML were based on stepwise regression of alleles selected based on ANOVA. The estimates from 2014 $F_{3:4}$ progeny row UHML were based on multiple regression of the alleles identified through stepwise regression.

§ Chromosomal assignments for each SSR primer pair correspond to the map position(s) specified in CottonGen (https://www.cottongen.org). N/A indicates that the SSR primers pairs had not been mapped a known linkage group.

Table 4. Multiple regression models of selected microsatellite marker (SSR) alleles associated with variation in fiber bundle strength (Str) within each population of F_3 individual plants and the resulting $F_{3:4}$ progeny rows when grown at College Station, TX, in 2013 and 2014, respectively.

Term†	F_3 estimate‡	$F_{3:4}$ estimate	Publication	Chr.§
Population A				
(04 WL-19/09207)				
Intercept	373.92***	348.70***		
BNL1454$_{100}$	**3.45****	**4.34****	Tan et al. 2015; Zhang et al. 2012; Yu et al. 2013a	15
BNL1604$_{98}$	**−6.05*****	**−6.81*****	Said et al. 2013	7, 16, 17
BNL2986$_{155}$	3.58***	0.62	Shen et al. 2006; Shen et al. 2007; Zeng et al. 2009	16
BNL3031$_{184}$	−5.49***	−0.50	He et al. 2007; Said et al. 2013	9, 23
BNL3545$_{183}$	**6.10*****	**4.91****	Wu et al. 2009	2, 14
C2–114$_{170}$	4.79***	1.89	Fang et al. 2014	7
DPL1071$_{245}$	−2.20*	−2.70	Kim et al. 2013	3
NAU1102$_{231}$	**10.28*****	**9.15*****	Cai et al. 2014; Shen et al. 2006	19
NAU1369$_{247}$	−3.00**	−2.87	Cai et al. 2014; Shen et al. 2006; Shen et al. 2007	8, 24, 25
NAU2581$_{257}$	**−6.37*****	**−6.29*****	Zhang et al. 2009	6
NAU3822$_{242}$	−2.65*	−1.17	Fang et al. 2014	N/A
Adjusted R^2	0.465	0.415		
Population B				
(*09 PP-03–02/09917*)				
Intercept	357.64***	345.81***		
BNL1604$_{98}$	**−4.94*****	**−4.05***	Said et al. 2013	7, 16, 17
BNL3400$_{175}$	4.87***	2.35	Fang et al. 2014; Qin et al. 2008	5, 19
CGR5139$_{182}$	3.32*	3.25	Wang et al. 2013a	16
NAU2291$_{206}$	−3.43**	−0.91	Fang et al. 2014; Said et al. 2013	4, 22
NAU3390$_{188}$	6.73**	0.11	Zhang et al. 2013	11
Adjusted R^2	0.174	0.053		
Population C				
(*TMC-9–2/LA 887*)				
Intercept	313.44***	318.76***		
BNL0686$_{157}$	5.16**	1.64	Said et al. 2013	23
BNL1604$_{120}$	4.82***	2.35	Said et al. 2013	7, 16, 17
BNL1605$_{76}$	3.26*	1.00	Zhang et al. 2012	12, 26
BNL1667$_{164}$	3.46*	2.80	Rong et al. 2004; Said et al. 2013	1, 2, 14, 15
BNL3031$_{184}$	7.13***	4.51	He et al. 2007; Said et al. 2013	9, 23, 26
CGR6329$_{232}$	**−3.71***	**−5.98****	Fang et al. 2014	26
CIR091$_{181}$	**−7.49*****	**−5.78***	Shen et al. 2007	4, 22
CIR246$_{157}$	−7.66***	−4.51	Qin et al. 2008; Wang et al. 2006	14
CIR249$_{186}$	**3.72****	**4.46***	Zeng et al. 2009	4
DPL1358$_{205}$	−9.25***	−1.93	Fang et al. 2014	11
HAU0087$_{188}$	6.88***	−0.24	Fang et al. 2014	4, 22
JESPR065$_{165}$	4.92*	2.40	Shen et al. 2006; Shen et al. 2007	5, 7, 21, 22, 26
NAU2291$_{197}$	**−5.17***	**−5.79***	Fang et al. 2014; Said et al. 2013	4, 22
NAU3393$_{196}$	**5.85*****	**3.86***	Sun et al. 2012; Wang et al. 2006	14
NAU4057$_{237}$	−4.97***	−0.06	Yu et al. 2013b	5
TMB0338$_{226}$	**5.54*****	**4.29***	Fang et al. 2014	25
Adjusted R^2	0.520	0.298		

* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level; alleles in bold were significantly associated with Str among the F_3 individual plants and $F_{3:4}$ progeny rows evaluated in 2013 and 2014, respectively.

† The 'present' allelic state was used as the base level for regression estimates for each allele. Positive or negative numbers indicate the direction of the associated response.

‡ The estimates from 2013 F_3 individual plant Str were based on stepwise regression of alleles selected based on ANOVA. The estimates from 2014 $F_{3:4}$ progeny row Str were based on multiple regression of the alleles identified through stepwise regression.

§ Chromosomal assignments for each SSR correspond to the map position(s) specified in CottonGen (https://www.cottongen.org). N/A indicates that the SSR primers pairs had not been mapped a known linkage group.

Upper-Half Mean Fiber Length

The first step of the analysis, ANOVA, identified 98 SSR alleles that were associated with UHML among the F_3 plants derived from all three populations, yet 55 of the alleles had opposite effects on UHML, depending on the genetic background, and were excluded from further analyses (data not shown). Thus, the effects of the remaining 43 alleles with consistent effects on UHML among the F_3 plants were estimated using stepwise multiple linear regression. The resulting stepwise model retained 15 SSR alleles that originated from five different publications, the majority of which used QTL mapping populations derived from diverse germplasms (Table 5). The publications included two studies that used upland intraspecific recombinant inbred lines (RILs) derived from a cross between strain 7235 and TM-1 that were evaluated in multiple environments (Shen et al., 2006, 2007), an association mapping study among lines derived from multiple crosses between upland and exotic cotton species, including *Gossypium barbadense*, *Gossypium tomentosum* Nutt ex Seem., *Gossypium. mustelinum*, and *Gossypium darwinii* Watt (Zeng et al., 2009), a meta-analysis of QTL mapping studies (Said et al., 2013), and a QTL mapping study using a random-mated RIL population derived from 11 diverse upland cotton cultivars (Fang et al., 2014).

The associations between the 15 SSR alleles and UHML of the F_3 plants were also measured among the $F_{3:4}$ progeny rows. Six alleles maintained a significant association with UHML across both years. The presence of $BNL1604_{98}$, $BNL4017_{234}$, $CGR5548_{162}$, and $CIR196_{192}$ was negatively associated with UHML, while the presence of $NAU1369_{247}$ and $NAU5046_{226}$ was positively associated with UHML. The majority of the SSRs had been previously mapped to two or more chromosomes. Still, the results suggest that the SSR alleles were associated with six separate QTL for UHML detected in all three populations, considering that none of the six SSRs had been mapped to the same chromosome, nor to homeologous chromosomes. For populations A and C, the mean UHML of $F_{3:4}$ progeny rows derived from F_3 plants having four to six alleles ($BNL1604_{98}$, $BNL4017_{234}$, $CGR5548_{162}$, $CIR196_{192}$, $NAU1369_{247}$, and $NAU5046_{226}$) in the desirable state (33.0 and 29.8 mm, respectively) was statistically equivalent to the mean of the $F_{3:4}$ progeny rows derived from F_3 plants in the top 20% for UHML, as measured by HVI (33.5 and 30.1 mm, respectively) (Table 6). In contrast, the mean UHML of $F_{3:4}$ progeny rows derived from

Table 5. Multiple regression models of selected microsatellite marker (SSR) alleles associated with variation in upper-half mean length (UHML) across populations of F_3 individual plants and the resulting $F_{3:4}$ progeny rows when grown at College Station, TX, in 2013 and 2014, respectively.

Term†	F_3 estimate‡	$F_{3:4}$ estimate	Publication	Chr.§
Intercept	31.51***	31.02***		
Population A (*04WL-19/09207*)	1.68***	1.14***		
Population B (*09 PP-03–02/09917*)	1.29***	0.94***		
$BNL0830_{104}$	−0.27***	0.02	Said et al. 2013	15
$BNL1604_{98}$	**−0.32*** **	**−0.27****	Said et al. 2013	7, 16, 17
$BNL2986_{155}$	−0.17*	−0.05	Shen et al. 2006; Zeng et al. 2009	16
$BNL4017_{234}$	**−0.17****	**−0.21***	Zeng et al. 2009	3, 14
$CGR5548_{162}$	**−0.21****	**−0.22***	Fang et al. 2014	20
$CIR196_{192}$	**−0.18****	**−0.20***	Zeng et al. 2009	11, 21
$DOW067_{162}$	0.22**	0.14	Fang et al. 2014	18
$DPL0270_{142}$	−0.18*	−0.11	Fang et al. 2014	11
$DPL0570_{302}$	0.13*	0.05	Fang et al. 2014	11
$JESPR050_{218}$	−0.22**	−0.17	Shen et al. 2007	5, 19, 22, 25
$MUSS422_{207}$	0.26***	0.16	Said et al. 2013	1, 15
$NAU2291_{206}$	−0.15*	−0.11	Fang et al. 2014; Said et al. 2013	4, 22
$NAU1369_{247}$	**0.17***	**0.18***	Shen et al. 2006; Shen et al. 2007	8, 24, 25
$NAU2265_{233}$	0.21**	0.07	Fang et al. 2014	2, 14
$NAU5046_{226}$	**0.16***	**0.20***	Fang et al. 2014	5, 22
Adjusted R^2	0.654	0.592		

* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level; alleles in bold were significantly associated with UHML among the F_3 individual plants and $F_{3:4}$ progeny rows evaluated in 2013 and 2014, respectively.

† Population C (*TMC-9–2/LA 887*) was used as the base level for regression estimates for population effects. The 'present' allelic state was used as the base level for regression estimates for each allele. Positive or negative numbers indicate the direction of the associated response.

‡ The estimates from 2013 F_3 individual plant UHML were based on stepwise regression of alleles selected based on ANOVA. The estimates from 2014 $F_{3:4}$ progeny row UHML were based on multiple regression of the alleles identified through stepwise regression.

§ Chromosomal assignments for each SSR primer pair correspond to the map position(s) specified in CottonGen (https://www.cottongen.org). N/A indicates that the SSR primers pairs had not been mapped a known linkage group.

Table 6. One-way analysis of variance and upper-half mean length (UHML) and fiber bundle strength (Str) of $F_{3:4}$ progeny rows based on marker-based and phenotypic grouping within three experimental populations.

	Population A (04 WL-19/09297)		Population B (09 PP-03–02/09917)		Population C (TMC-9–2/LA 887)	
UHML						
Source	**df**	**MS†**	**df**	**MS**	**df**	**MS**
Grouping method‡	1	2.51	1	5.41*	1	1.23
Error	50	1.56	39	1.24	37	1.45
Mean UHML	**N**	**mm**	**N**	**mm**	**N**	**mm**
Marker-based	26	33.0 a§	16	32.8 b	14	29.8 a
Phenotypic	26	33.5 a	25	33.6 a	25	30.1 a
Str						
Source	**df**	**MS**	**df**	**MS**	**df**	**MS**
Grouping method‡	1	47.33	1	2.95	1	0.01
Error	55	354.72	66	484.88	37	296.82
Mean Str	**N**	**kN m kg^{-1}**	**N**	**kN m kg^{-1}**	**N**	**kN m kg^{-1}**
Marker-based	42	356.13 a§	58	355.61 a	25	332.60 a
Phenotypic	15	358.20 a	10	356.20 a	14	332.63 a

*Significant at the 0.05 probability level.

† MS, Mean squares.

‡ The mean UHML and Str of $F_{3:4}$ progeny rows derived from F_3 plants having four-to-six alleles in the desirable state (i.e. marker-based grouping) were compared with those of $F_{3:4}$ progeny rows derived from F_3 plants in the top 20% for UHML and Str, as measured by high-volume instrument (HVI) (i.e. phenotypic grouping).

§ Means within columns (i.e. within population) with the same letters are not significantly different at the 0.05 probability level according to Fisher's protected least significant difference.

F_3 plants in the top 20% for UHML (33.6 mm) was greater than the mean of the $F_{3:4}$ progeny rows derived from F_3 plants having four to six alleles in the desirable state (32.8 mm) for population B.

Fiber Bundle Strength

Analysis of variance identified 104 SSR alleles that were associated with Str among the F_3 plants, 62 of which were associated with opposite effects on Str, depending on the genetic background, and were excluded from further analyses (data not shown). Stepwise linear regression of Str among the F_3 plants was conducted based on the remaining 42 SSR alleles associated with consistent effects across the three populations. The resulting model retained 17 alleles having an association with Str among the F_3 plants (Table 7). The set of 17 SSRs originated from the five studies listed in the previous section on UHML (Shen et al., 2006, 2007; Zeng et al., 2009; Said et al., 2013; Fang et al., 2014) plus three additional studies, including an association analysis of fiber quality traits among 99 upland cotton cultivars and accessions (Cai et al., 2014) and two QTL analyses within upland intraspecific populations Yumian 1/T586 (Zhang et al., 2005) and CCRI 35/Yumian 1 (Tan et al., 2015).

The associations between the 17 alleles and Str were also measured among the $F_{3:4}$ progeny rows grown in 2014. Only six of the SSR alleles maintained a significant association with 2014 Str. The presence of $BNL1604_{98}$, $CGR6329_{232}$, $DPL0236_{157}$, and $NAU1369_{247}$ was negatively associated with Str, while the presence of $NAU1102_{231}$ and $TMB0382_{179}$ was positively associated with Str. $DPL0236_{157}$ had not been mapped to a linkage group or chromosome position in the original publication (Fang et al., 2014). Among the five remaining SSRs, no two had previously been mapped to the same chromosome, nor to homeologous chromosomes; thus, it is plausible that the five markers were linked to separate QTL for fiber Str present in each of the three populations. For populations A, B, and C, there was no significant difference between the mean Str of $F_{3:4}$ progeny rows derived from F_3 plants having four to six alleles ($BNL1604_{98}$, $CGR6329_{232}$, $DPL0236_{157}$, $NAU1369_{247}$, $NAU1102_{231}$, and $TMB0382_{179}$) in the beneficial state (356.13, 355.61, and 332.60 kN m kg^{-1}, respectively) and the mean of the $F_{3:4}$ progeny rows derived from F_3 plants in the top 20% for Str, as measured by HVI (358.20, 356.20, and 332.63 kN m kg^{-1}, respectively) (Table 6).

Fiber Quality QTL Clusters

Several SSR alleles identified through the two-step analysis across populations were associated with both UHML and Str. Numerous studies have reported the tendency for QTL of various fiber quality traits to colocalize, or form QTL 'clusters' (Rong et al., 2007; Lacape et al., 2010; Said et al., 2013). There was a positive phenotypic correlation between UHML and Str across populations, though this association was weak to nonexistent within the populations with superior fiber quality (populations A and B) (Table 8). Still, it is unclear whether the SSR alleles are associated with separate colocalizing QTL or a single QTL with pleiotropic effects. BNL0830, JESPR050, and NAU2291 were significantly associated with UHML and Str among the F_3 plants but not in the $F_{3:4}$ progeny rows, while BNL1604 and NAU1369 were significantly associated with UHML and Str across generations. The presence of $NAU1369_{247}$ was negatively associated with Str but positively associated with UHML. Shen et al. (2006, 2007) reported that NAU1369 was linked to a QTL for Str on chromosome 25, originating from upland germplasm line 7235, which contains alleles introgressed from *Gossypium anomalum* Waw. et Peyr. This QTL on chromosome 25 explained from 5.6 to 11.7% of the variation in Str. Qin et al. (2008) mapped NAU1396 to chromosome 24, but did not detect any association between the SSR marker and Str. Still, multiple studies suggest that chromosome 24 harbors multiple QTL for Str (Chen et al., 2009; Kumar et al., 2012). Unfortunately, we were unable to estimate the location of this QTL within the three populations, due to the missing parental genotypes.

Table 7. Multiple regression models of selected microsatellite marker (SSR) alleles associated with variation in fiber bundle strength (Str) across populations of F_3 individual plants and the resulting $F_{3:4}$ progeny rows when grown at College Station, TX, in 2013 and 2014, respectively.

Term†	F_3 estimate‡	$F_{3:4}$ estimate‡	Publication	Chr.§
Intercept	348.73***	336.14***		
Population A (*04WL-19/09207*)	13.23***	6.61***		
Population B (*09 PP-03–02/09917*)	15.37***	8.88***		
$BNL0830_{104}$	–3.02***	–1.27	Said et al. 2013	15
$BNL1122_{166}$	2.37**	–0.60	Shen et al. 2005; Shen et al. 2007; Zeng et al. 2009; Cai et al. 2014	7, 16
$BNL1604_{120}$	2.76*	0.80	Said et al. 2013	7, 16, 17
$BNL1604_{98}$	**–3.63****	**–3.97***	Said et al. 2013	7, 16, 17
$BNL2599_{96}$	–2.83***	–0.97	Fang et al. 2014	1
$BNL3280_{213}$	–2.05*	0.89	Zhang et al. 2005	18, 20
$CGR6329_{232}$	**–2.43****	**–2.71***	Fang et al. 2014	26
$CIR249_{192}$	–2.74**	–1.96	Zeng et al. 2009	4
$DPL0236_{157}$	**–1.91***	**–2.80****	Fang et al. 2014	N/A
$DPL1358_{205}$	–3.14***	–1.86	Fang et al. 2014	11
$JESPR050_{218}$	–2.17*	–0.74	Shen et al. 2007	5, 19, 22, 25
$JESPR295_{105}$	2.94***	1.87	Cai et al. 2014	12, 26
$NAU2291_{203}$	3.56***	0.47	Fang et al. 2014; Said et al. 2013	4, 22
$NAU1102_{231}$	**2.21***	**2.85***	Cai et al. 2014	19
$NAU1369_{247}$	**–2.71*****	**–2.69****	Shen et al. 2006; Shen et al. 2007	8, 24, 25
$TMB0382_{179}$	**1.96***	**2.48***	Tan et al. 2015	23
$UCD120_{-266}$	1.62*	0.73	Fang et al. 2014	22
Adjusted R^2	0.535	0.375		

* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level; alleles in bold were significantly associated with Str among the F_3 individual plants and $F_{3:4}$ progeny rows evaluated in 2013 and 2014, respectively.

† Population C (*TMC-9–2/LA 887*) was used as the base level for regression estimates for population effects. The 'present' allelic state was used as the base level for regression estimates for each allele. Positive or negative numbers indicate the direction of the associated response.

‡ The estimates from 2013 F_3 individual plant Str were based on stepwise regression of alleles selected based on ANOVA. The estimates from 2014 $F_{3:4}$ progeny row Str were based on multiple regression of the alleles identified through stepwise regression.

§ Chromosomal assignments for each SSR correspond to the map position(s) specified in CottonGen (https://www.cottongen.org). N/A indicates that the SSR primers pairs had not been mapped a known linkage group.

Table 8. Correlation between upper-half mean fiber length (UHML) and fiber bundle strength (Str) among F_3 progeny plants and $F_{3:4}$ progeny rows grown at College Station, TX, in 2013 and 2014, respectively.

	Correlation coefficient†	
	F_3	$F_{3:4}$
Combined	0.6353***	0.4939***
Population A (*04WL-19/09207*)	0.1757**	0.0828
Population B (*09 PP-03–02/09917*)	0.2471***	0.0886
Population C (*TMC-9–2/LA 887*)	0.6035***	0.6118***

* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level.

† Pearson's correlation coefficient was calculated to estimate the phenotypic correlation between fiber UHML and Str.

The absence of $BNL1604_{98}$ was positively associated with both UHML and Str, but it is not an ideal candidate for MAS, considering that the BNL1604 primer pair amplified more than one locus in all three populations. In fact, BNL1604 has been mapped to multiple linkage groups, including homeologous chromosomes 7 (Zhang et al., 2012) and 16 (Wang et al., 2011), as well as chromosome 17 (Song et al., 2005). Wang et al. (2011) reported that BNL1604 was linked to a QTL for UHML and micronaire on chromosome 16 within an interspecific (*G. hirsutum* × *G. barbadense*) population. Mapping studies among upland intraspecific populations suggest that BNL1604 is associated with a fiber quality QTL cluster on chromosome 7, which includes QTL for UHML, Str, micronaire, length uniformity, and elongation (Sun et al., 2012; Zhang et al., 2012; Said et al., 2013; Yu et al., 2013b; Tan et al., 2015).

CONCLUSIONS

The results reported herein provide further evidence that UHML and Str are controlled by a complex network of interacting genes of relatively small effect (Meredith, 1984) and demonstrate the importance of QTL × genetic background interactions. In the combined analysis across populations, over half of the alleles were associated with opposite effects on UHML and Str, depending on the genetic background. Either epistasis plays a substantial role in the phenotypic expression of UHML and Str, or the majority of SSRs utilized in this study are not in tight enough linkage disequilibrium with QTL for UHML and Str to be portable across genetic backgrounds.

Nevertheless, despite the challenges of comparing QTL mapping results across different studies, six previously reported SSR–UHML associations ($BNL1604_{98}$, $BNL4017_{234}$, $CGR5548_{162}$, $CIR196_{192}$, $NAU1369_{247}$, and $NAU5046_{226}$) and six SSR–Str associations ($BNL1604_{98}$, $CGR6329_{232}$, $DPL0236_{157}$, $NAU1102_{231}$, $NAU1369_{247}$, and $TMB0382_{179}$) were detected in the two-step analysis across populations. A number of these marker–trait associations were also identified in the two-step analysis within one or more populations, including BNL1604, CGR6329, NAU1102, and NAU1369. These results provide further support that QTL studies that utilize diverse genetic backgrounds are most effective at identifying genetic markers linked to stable QTL for fiber quality, compared with QTL mapping within biparental populations (Zeng et al., 2009; Said et al., 2013; Cai et al., 2014; Fang et al., 2014).

Using the 12 SSR alleles identified through the two-step analysis across populations, the mean UHML and Str of $F_{3:4}$ progeny rows derived from F_3 plants having four to six of the alleles in the desirable state was equivalent to the mean of the $F_{3:4}$ progeny rows derived from F_3 plants in the top 20% for both UHML and Str, with only one exception—UHML within population B. Even though the variation in fiber quality for each population was comparable, there were fewer marker–trait associations identified for UHML and Str among the F_3 progeny within population B. These data may suggest that population B harbors unique QTL for UHML and Str that had not been previously characterized in the mapping publications used in this study.

While marker-based selection using genetic markers linked to QTL for fiber quality is not likely to serve as a substitute for phenotypic selection, our results suggest that markers linked to stable QTL for UHML and Str could make the simultaneous improvement of fiber quality and lint yield through MAS more efficient. Genotypic information regarding fiber quality traits could be used in the selection of parental lines or selection of progeny before planting or phenotypic evaluation, increasing the probability of identifying rare recombinants with improved fiber quality and lint yield. Future studies that involve replicated testing of advanced germplasm lines developed through MAS will provide greater insight into the efficacy of molecular breeding methods for the improvement of fiber quality traits.

Supplemental Material Available

Supplemental material for this article is available online.

Conflict of Interest

The authors declare that there is no conflict of interest.

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