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RESEARCH

Genetic Evaluation of Exotic Chromatins from Two Obsolete Interspecific Introgression Lines of Upland Cotton for Fiber Quality Improvement

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ABSTRACT

Interspecific introgression lines are important resources for plant breeders to access novel alleles from exotic germplasm. The Pee Dee breeding program developed several extra-long staple "Sealand" germplasm lines that presumably contain sea island cotton (*Gossypium barbadense* L.) chromatin introgressed into upland cotton (*Gossypium hirsutum* L.). The objectives of this study were to identify the *G. barbadense* chromatins in the Sealand lines, 542 and 883, and evaluate their effects on fiber quality. A total of 31 putative *G. barbadense* chromatins were detected, including 12 segments on seven chromosomes that were unique to Sealand 542 and 10 segments on five chromosomes that were unique to Sealand 883, and the remaining nine segments were common to both the Sealand lines. Sealand 542 and 883 mapping populations were created to identified 28 quantitative trait locus (QTL) regions associated with fiber quality traits, including six for elongation, four for fineness, five for short fiber content, five for strength, three for length, and five for fiber uniformity. At least 13 QTLs were detected on the *G. barbadense* introgressed chromosomal segments. Favorable alleles for 17 QTLs were contributed by the Sealand lines, and the genetic effects of five loci were stably expressed across environment and generation. Stable expression of *G. barbadense* fiber quality alleles in the Sealand lines and the absence of these alleles in upland germplasm make these introgression lines a valuable resource for fiber quality improvement. In particular, marker-assisted introgression of the *qFL-Chr25* locus from Sealand 883 should result in improved fiber length, whereas that of *qMIC-Chr24* from Sealand 542 should result in finer fiber.

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Abbreviations: GRIN, Germplasm Resources Information Network; LOD, logarithm of odds; PCR, polymerase chain reaction; PV%, percentage phenotypic variation; QTL, quantitative trait locus; SSR, simple sequence repeat.

Despite having greater productivity, genetic improvement of yield and fiber quality traits in upland cotton (*Gossypium hirsutum* L.) is generally slow and faces many challenges. First is the negative association between fiber quality traits and yield components (Culp and Green, 1992). Since yield improvement has been the foremost objective of a breeding program, it is challenging for a breeder to target fiber quality without compromising lint yield. Second is the low genetic diversity in the *G. hirsutum* gene pool due to genetic bottlenecks imposed during polyploidization about 1 to 2 million yr ago and its subsequent domestication from a small subset of its wild ancestors (Paterson et al., 2004). In addition, the traditional approach in upland cotton improvement has relied on crossing lines from a few genetic backgrounds and reselection within the population for yield and fiber quality traits (May et al., 1995; Van Esbroeck and Bowman, 1998; Gingle et al., 2006). Together these events resulted in a cultivated Upland germplasm with a narrow genetic base, which constrains future genetic improvement.

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Large amount of genetic variation exist in the secondary and tertiary gene pools of *Gossypium* (Percival and Kohel, 1990; Lacape et al., 2007; Lubbers and Chee, 2009; Percy, 2009). In particular, *Gossypium barbadense* L. (also known as pima, sea island, or Egyptian cotton) is the second most widely cultivated species of cotton and has superior fiber quality traits such as longer, stronger, and finer fibers than upland cottons. Transferring the superior fiber quality traits of *G. barbadense* into upland germplasm is attractive but has been an elusive objective. Typical obstacles faced in introgressive breeding are hybrid breakdown (Stephens, 1949), distorted genetic segregation in early generations (Reinisch et al., 1994) and in advanced generations (Jiang et al., 2000), linkage drag, and a high level of epistasis between fiber quality quantitative trait loci (QTLs) and genetic backgrounds harboring different unlinked introgressed alleles (Chee et al., 2005a, 2005b). Historical accounts indicated that the upland germplasm has substantial exposure to both gene pools through intentional breeding efforts (Ware, 1951; Culp and Harrell, 1975; Lubbers and Chee, 2009). However, the extent of the genetic contribution of wild donors in upland germplasm is largely unknown because evidence of putative interspecific introgression into upland cotton can only be detected in loci that are fixed or nearly fixed in secondary gene pools but are absent or rare in upland cotton germplasm (Lubbers and Chee, 2009). Thus, although pedigree analyses indicated that many cultivars were developed through interspecific hybridization, molecular analysis based on isozymes and DNA markers on the upland cotton germplasm indicated that the genepool is largely homogeneous. Rare alleles, which have likely arisen through introgression, are restricted to only a few closely related cultivars within a germplasm group (Wendel et al., 1992; Brubaker and Wendel, 1994; Iqbal et al., 2001). These results suggest that the secondary and tertiary gene pools remains largely unused in upland cotton improvement.

Nonetheless, some examples have documented the successful use of the secondary gene pool in developing upland cotton cultivars. In the 1930s, the cultivated sea island cotton (*G. barbadense*) was used to develop extra-long staple cottons (Ware, 1951; Smith and Cothren, 1999), including the series of "Sealand" germplasm lines developed by the USDA-ARS Pee Dee breeding program in Florence, SC (Jenkins, 1948). These Sealand cultivars have improved fiber length and fineness compared with upland cultivars available during that time (Campbell et al., 2011). Cultivars 'Sealand 542' and 'Sealand 883' were commercially grown on ~400 ha in South Carolina, Georgia, and Florida until the 1950s (Culp and Harrell, 1974).

The significantly longer and finer fibers of Sealand cultivars compared with their recurrent upland parent suggest that they contain stable introgressions of genes conferring these traits from the *G. barbadense* donor parent. However, the extent of introgression, chromosome locations, and genetic effects of the introgressed chromatin are largely unknown. Our objectives in this study were to identify the genomic position of the introgressed chromatin in Sealand 542 and Sealand 883 and to evaluate genetic effects of the introgressed alleles on fiber quality traits. Detailed genetic dissection of the introgressed regions offer the opportunity to better understand the genetic effects of backcross introgression involving interspecific hybridization and to provide tools for selecting rare introgressed progenies that carry desirable combinations of genes to further improve cotton fiber quality.

MATERIALS AND METHODS

Plant Material and Phenotypic Measurements

Two F_2 populations designated as Pop-542 and Pop-883 were generated by crossing an upland cotton line Suyuan 7235 as a female parent with Sealand 542 (PI 528730) and Sealand 883 (PI 528875), respectively. The germplasm line Suyuan 7235 (referred to herein as S-7235) was developed by the Jiangsu Academy of Agricultural Sciences, China (Qian et al., 1992). The Sealand 542 and 883 parents (referred to herein as SL-542 and SL-883, respectively) are cultivars developed at the USDA-ARS Pee Dee Experiment Station in the 1930s. Sealand 542 and 883 are sister lines developed from a common interspecific cross between the *G. barbadense* cultivar 'Bleak Hall' (PI 608115) and the upland cotton cultivar 'Coker Wilds' (Culp and Harrell, 1974; Bowman et al., 2006), followed by four backcrosses to the upland cotton recurrent parent.

F_1s of the two crosses were grown in the greenhouse, and F_2 seeds were collected from a single plant in both crosses. Over 350 F_2 seeds from each of the two crosses were planted at the William Gibbs Farm on the University of Georgia–Tifton campus in Tifton, GA, in the summer of 2005. One hundred and seventy-five individuals were randomly tagged in each of the two F_2 populations, and seeds were harvested from these tagged individuals. In 2006, 175 $F_{2:3}$ families along with the three parents were planted as progeny rows in a completely randomized design with two replications at the same farm in Tifton. All the 175 $F_{2:3}$ families were advanced to the $F_{2:4}$ generation in 2007, where they were again planted together with the three parents in a completely randomized design with two replications in Tifton. The plots were single-row plots, 9 m by 1 m, planted at four seeds per row foot in early May and harvested in early October. Standard production practices were followed in each test.

All open cotton bolls were harvested from individual F_2 plants, and a 25-boll sample was harvested from each $F_{2:3}$ and $F_{2:4}$ progeny row, ginned on a table-top saw gin, and tested for fiber quality using the high volume instruments at the Cotton Incorporated Textile Services Laboratory (Cotton Incorporated, Cary, NC). The fiber quality measurements included upper half mean length in milliliters, fiber strength in kilonewton meters per kilogram (kN m kg^{-1}), where 1 N equals 9.81 kg force, fiber fineness or micronaire, percentage fiber elongation, percentage short fiber content, and percentage uniformity index.

DNA Preparation and Molecular Marker Analysis

Genomic DNA of the parents and F_2 plants from both mapping populations was extracted following a published protocol (Paterson et al., 1993). Quantity and quality of the extracted DNA was determined by running the samples on a 0.8% agarose gel. The concentration of genomic DNA was estimated by comparing the size and intensity of each sample band with those of the standard λ uncut DNA (1 μg 5 μL^{-1}) and commercial MassRuler (Thermo Fisher Scientific).

Polymerase chain reaction (PCR) conditions were slightly modified from the previously described protocol (Chee et al., 2004). Polymerase chain reaction amplification was performed in a PTC-100 or PTC-200 thermocycler (MJ Research). A 10-μL reaction contained 10 ng of template DNA, 0.5 μM primer mix, 100 μM deoxynucleotides, 1.5 mM $MgCl_2$, 3 U of DNA polymerase, and 1× reaction buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl). The cycling conditions for PCR were 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.2 min. After the last cycle, reactions were incubated at 72°C for 6 min before cooling to 4°C. For primer pairs that did not amplify in the first two attempts, a gradient reaction from 45 to 60°C was performed to empirically determine the best annealing temperature (Chee et al., 2004). The amplified PCR products were electrophoretically separated on a 10% nondenaturing polyacrylamide gel electrophoresis (PAGE) and were visualized by staining with $AgNO_3$ following the published procedures (Zhang et al., 2002).

The polymorphism survey panel was genotyped with a total of 1170 simple sequence repeat (SSR) markers, covering all of the 26 homeologs. Sequences of the SSR markers were downloaded from the Cottongen database (www.cottongen.org) and were commercially synthesized by Eurofins MWG Operon (Huntsville, AL). Polymorphic markers were considered evidence of *G. barbadense* chromatin transferred during the development of the Sealand 542 and 883 cultivars. Thus, differences with SL-542, SL-883, or both from the common S-7235 were classified as (i) unique polymorphism (or introgression) in SL-542, (ii) unique polymorphism (or introgression) in SL-883, and (iii) *G. barbadense* introgressed common to both SL-542 and SL-883.

Genetic Mapping and QTL Analysis

The phenotypic distributions of fiber quality traits and the Pearson correlation coefficients among the traits in F_2, $F_{2:3}$, and $F_{2:4}$ of both populations were calculated using PROC UNIVARIATE and PROC CORR procedures of SAS version 9.1 (SAS Institute, 1989), respectively. Broad sense heritability (H^2) of the fiber traits, calculated from the ratio of total genetic variance to total phenotypic variance, was estimated by parent–offspring regression of 175 $F_{2:3}$ to $F_{2:4}$ in each population.

Linkage groups were constructed by using Mapmaker/EXP 3.0 (Lander et al., 1987) software. The assembly of the linkage groups was done using the 'group' command with a logarithm of odds (LOD) score of 3.0 and a maximum recombination fraction of 30 cM. Recombination units were converted into genetic distances by using the Kosambi mapping function (Kosambi, 1944) with the "error detection" command on. Unlinked markers were added to the framework using the "try" and "compare" commands. The final order of marker sequence on a linkage group was confirmed using the "ripple" command. Assignment of linkage groups to the chromosome and subgenome is based on the published comprehensive reference map of tetraploid cotton (Yu et al., 2010).

Detection of QTLs and estimation of various genetic parameters were performed by the composite interval mapping function implemented in the software WinQTL Cartographer version 2.5 (Wang et al., 2005). The likelihood ratio threshold value ($\alpha = 0.05$) for declaring the presence of QTL was estimated after 1000 permutations (Doerge and Churchill 1996). Peaks below this threshold but with a LOD >2.5 were considered putative QTLs. Mapping was performed at 1-cM walk speed in a 10-cM window with five background cofactors, where the cofactors were selected via forward–backward stepwise regression method. Quantitative trait loci were defined by one-LOD confidence intervals on either sides of the peak position and were named following a method used in rice (*Oryza sativa* L.; McCouch et al., 1997). Briefly, the QTL is designated as "*q*" followed by an abbreviation of the trait name, which is then followed by the chromosome name. Multiple QTLs on the same chromosome are distinguished by an alphabetical suffix. For brevity, we classified QTLs as "common" when a QTL is detected in both populations, "consistent" if a QTL is detected in all three (F_2, $F_{2:3}$, and $F_{2:4}$), generations and "unique" if detected in only one generation of any population.

RESULTS

Linkage Maps and Putative Introgressed Regions

Among the 1170 SSR loci surveyed, 165 loci were polymorphic, including 45 unique to Pop-542, 40 unique to Pop-883, and 80 loci polymorphic in both populations. Genetic mapping positioned the 125 (45 unique + 80 shared) polymorphic SSR loci to 21 putative introgressions segments on 15 chromosomes in Pop-542. Twelve segments on seven chromosomes (3, 5, 6, 9, 18, 21, and 23) were unique to Pop-542, and the remaining nine segments were also mapped in Pop-883. Similarly, 120 (40 unique + 80 shared) polymorphic SSR markers mapped to 19 introgressed segments on 13 chromosomes in Pop-883. The 10 segments unique to Pop-883 mapped to five chromosomes (5, 11, 15, 16, and 25). The recombination length of all segments covered 530 and 411 cM in Pop-542 and Pop-883, respectively. Unique introgressed regions covered 351 cM (6%) of the cotton genome in SL-542 and 235 cM (4%) in SL-883. The map locations of each introgressed segment in SL-542 and SL-883 were plotted on a comprehensive reference map of the tetraploid cotton genome (Yu et al., 2010) (Fig. 1). Eighty marker loci were polymorphic in both Pop-542 and Pop-883, mapping to nine genomic regions on eight chromosomes. These genomic regions may represent genetic variation (polymorphism) between S-7235 and the recurrent upland parent, 'Coker Wilds', of the

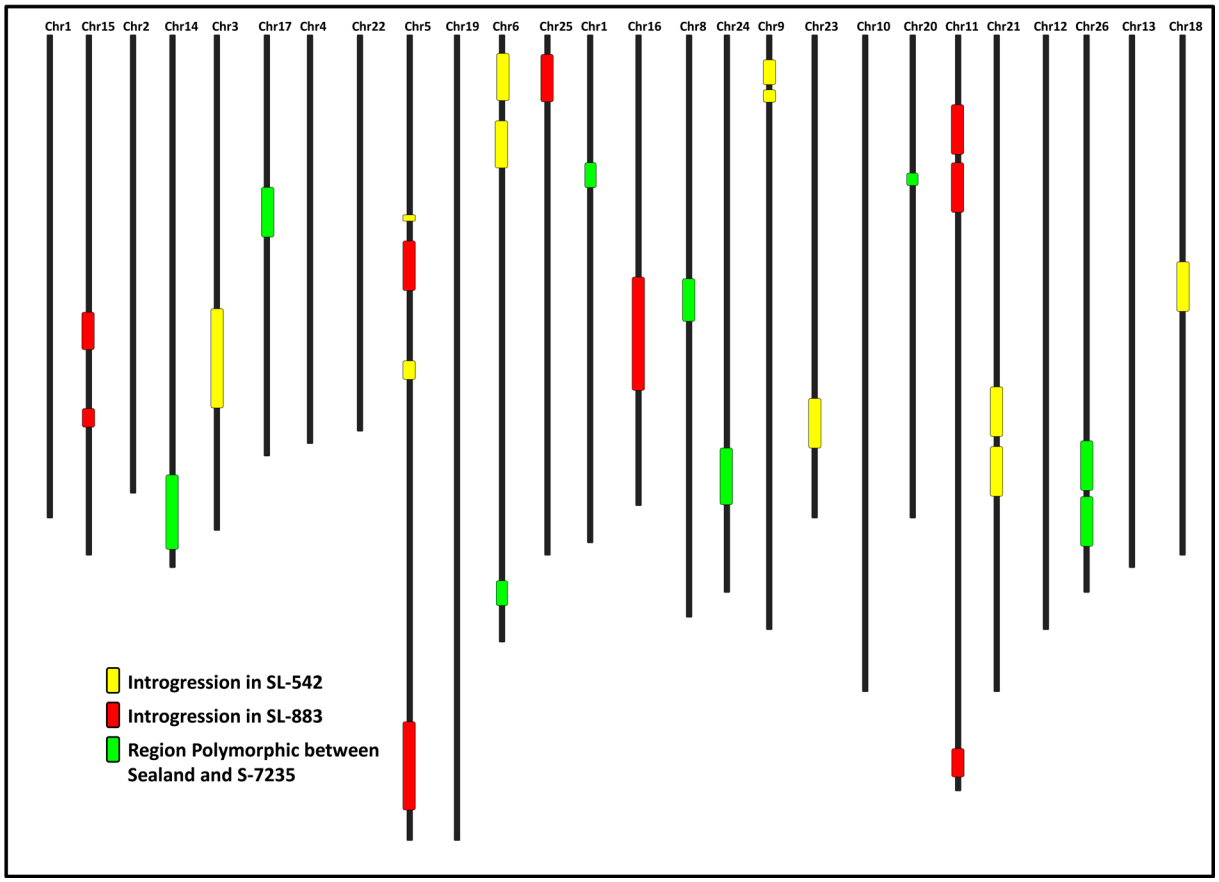

Fig. 1. Tentative locations of the introgressed segments plotted on the genome-wide comprehensive reference map of tetraploid cotton by Yu et al. (2010).

Sealands pedigree, but *G. barbadense* introgressions in both SL-542 and SL-883 is a more probable interpretation. Given that SL-542 and SL-883 are sister cultivars selected from the same pedigree, it would be expected that *G. barbadense* segments are common.

Population Biometric Parameters

Both SL-542 and SL-883 possessed fiber quality traits that were different from S-7235 (Fig. 2). Between the three parents, SL-542 had the greatest fiber elongation and fineness, whereas SL-883 had the greatest fiber length. SL-542 and SL-883 had lower fiber strength and fiber uniformity but greater short fiber content than the S-7235 (Fig. 2). Continuous variation for all fiber traits was observed in advanced generations (both $F_{2:3}$ and $F_{2:4}$) of Pop-542 and Pop-883, supporting the polygenic inheritance of these traits. The population mean was close to the mid-parent value for all traits except for fineness in Pop-542 and fiber length in Pop-883, where it was skewed towards the lower value parent (S-7235) (Fig. 2) Transgressive segregation was observed for most of the fiber quality traits analyzed.

Pearson correlation coefficients between fiber quality traits, calculated for all six datasets, showed strong positive correlation between fiber length, fiber strength, and uniformity index (Table 1) in all three of the pairwise comparisons. Fiber length showed significant negative correlation with fiber elongation, fiber fineness, and short fiber content. Very strong negative correlations (up to $r = -0.88$) were detected between short fiber content and uniformity index. Early generation analysis of a quantitative trait is appropriate, especially when the trait shows relatively high heritability. By performing $F_{2:3}$ to $F_{2:4}$ regressions, we determined that all fiber quality traits had moderate to high heritability estimates ranging from 0.38 to 0.50, except for fiber uniformity, where it ranged from 0.16 to 0.30 (Table 2). The range of H^2 values estimated from the parent–offspring regressions were in congruence with the earlier estimates of narrow-sense heritability (May, 1999) and support the validity of an early-generation study (Paterson et al., 2003).

QTL Analysis

A total of 28 QTLs affecting fiber quality were detected in both populations by composite interval mapping (Table 3). Twelve QTLs were identified on A subgenome chromosomes, whereas 16 were identified on D subgenome chromosomes (Fig. 3). Seven QTLs were unique to Pop-542, and 16 were unique to Pop-883, whereas five QTLs were common between the two populations. Details of the QTLs identified for each fiber quality traits are summarized below.

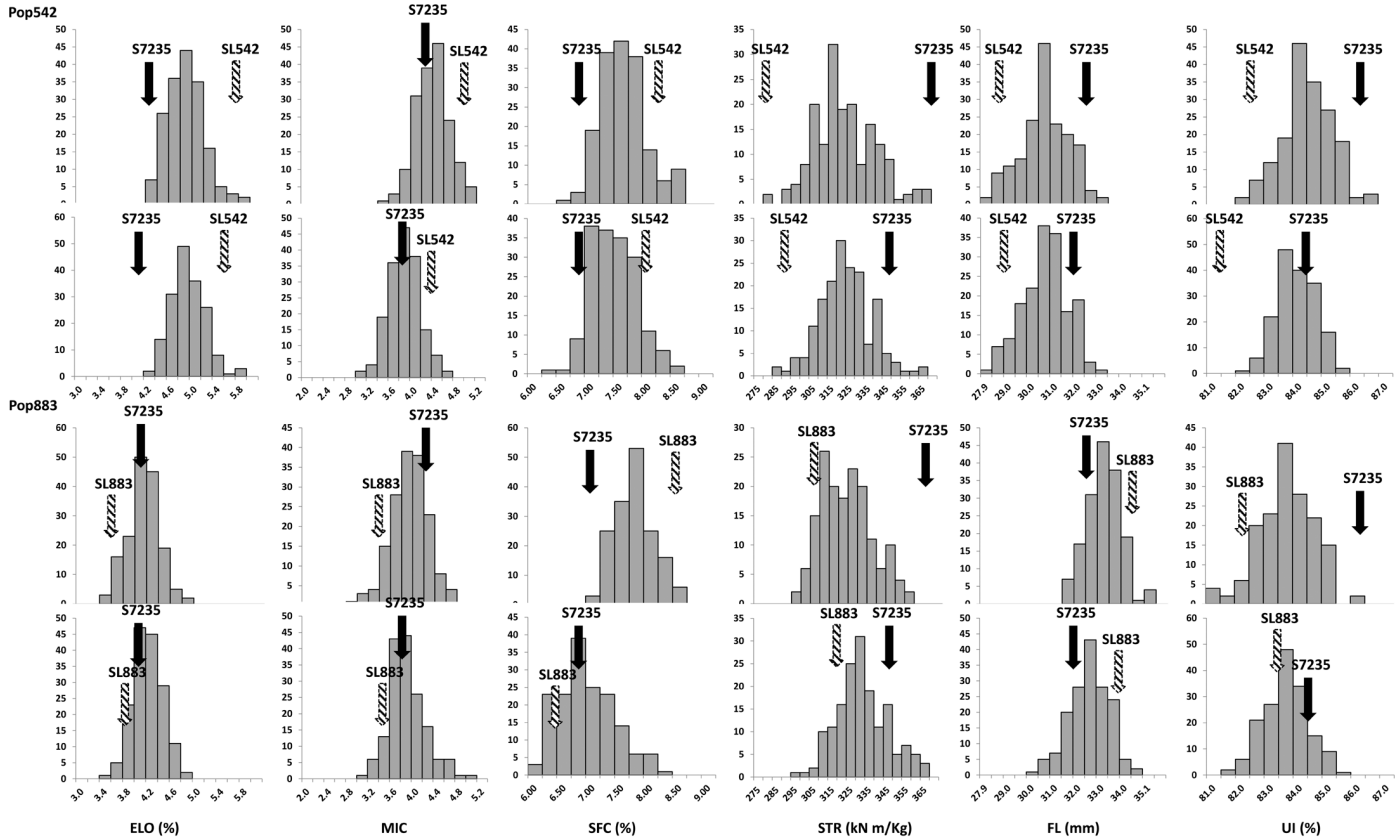

Fig. 2. Distribution of fiber quality $F_{2:3}$ and $F_{2:4}$ generation of the Pop542 and Pop883 mapping populations. ELO, elongation; MIC, micronaire (fineness): SFC, short fiber content; STR, strength; FL, upper half mean length; UI, uniformity index.

Fiber Elongation

A total of six QTLs were identified. Three (*qELO-Chr08*, *qELO-Chr21*, and *qELO-Chr23*) were specific to Pop-542, and two (*qELO-Chr5* and *qELO-Chr11*) were specific to Pop-883 (Table 3). The QTLs *qELO-Chr11* and *qELO-Chr23* had significant phenotypic effects in all three tested generations in both populations. A common QTL, *qELO-Chr24*, was identified in at least one generation in both populations. The percentage phenotypic variation (PV%) explained by a QTL ranged from 2.0 to 13.6%, with the genotypic effects ranging from 0.1 to 0.8%. The Sealand parents contributed favorable alleles for the consistent QTLs *qELO-Chr11*, *qELO-Chr21*, and *qELO-Chr23*, whereas S7235 contributed favorable alleles for *qELO-Chr05* and *qELO-Chr24*.

Table 1. Pearson correlation coefficients among fiber quality traits in three generations of Pop542 and Pop883 (numbers after slash).

Trait	Generation	Fineness	Short fiber content	Fiber strength	Uniformity index	Fiber length
Fiber elongation	F_2	0.05\0.16	0.09\-0.15	−0.19**\−0.05	−0.10\0.16**	−0.18\−0.31***
	$F_{2:3}$	0.36***\0.38***	−0.09\0.04	−0.08\0.13	0.06\0.23**	−0.29***\−0.30***
	$F_{2:4}$	0.15\0.40***	0.09\-0.32***	−0.18\0.30***	−0.03\0.24***	−0.30***\−0.13
Fiber fineness	F_2		−0.19**\−0.34**	0.30***\0.33***	0.22**\0.50	−0.31***\−0.15**
	$F_{2:3}$		0.03\0.20	0.04\0.18	0.01\0.20**	−0.41***\−0.37***
	$F_{2:4}$		−0.10\−0.47***	0.03\0.42***	0.20**\0.48***	−0.29***\−0.01
Short fiber content	F_2			−0.42***\−0.43**	−0.80***\−0.79***	−0.47***\−0.35***
	$F_{2:3}$			−0.54***\−0.47***	−0.87***\−0.46***	−0.57***\−0.85***
	$F_{2:4}$			−0.46***\−0.58***	−0.78***\−0.88***	−0.71***\−0.41***
Fiber strength	F_2				0.47***\0.54***	0.31***\0.30***
	$F_{2:3}$				0.57***\0.45***	0.52***\0.35***
	$F_{2:4}$				0.48***\0.56***	0.41***\0.33***
Uniformity index	F_2					0.53***\0.27***
	$F_{2:3}$					0.56***\0.23***
	$F_{2:4}$					0.44***\0.29***

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 2. Parent–offspring broad-sense heritability estimates of fiber quality traits for Pop542 and Pop883.

Trait	Population	H^2 estimate
Fiber elongation	Pop542	0.38
	Pop883	0.45
Fiber Fineness	Pop542	0.50
	Pop883	0.50
Short fiber content	Pop542	0.38
	Pop883	0.35
Fiber strength	Pop542	0.41
	Pop883	0.49
Fiber length	Pop542	0.50
	Pop883	0.45
Fiber uniformity	Pop542	0.16
	Pop883	0.30

Fiber Fineness

A total of four QTLs were identified. Two (*qMIC-Chr11* and *qMIC-Chr17*) were unique to Pop-883, whereas one (*qMIC-Chr08*) was unique to Pop-542 (Table 3). A consistent QTL, *qMIC-Chr17*, was detected in all the three generations of Pop-883, although it was detected as a putative locus in the $F_{2:4}$ generation. The QTL *qMIC-Chr24* is common and consistent and was identified in all three generations of both mapping populations. The PV% explained by these QTLs ranged from 2.4 to 33.6%, with genetic effects ranging from 0.1 to 0.4 units. SL-542 and SL-883 contributed favorable alleles for *qMIC-Chr09* and *qMIC-Chr11* respectively, whereas S-7235 contributed favorable alleles for *qMIC-Chr17* and *qMIC-Chr24*.

Short Fiber Content

A total of five QTLs were identified. Four (*qSFC-Chr05*, *qSFC-Chr08*, *qSFC-Chr17*, and *qSFC-Chr245*) were unique to Pop-883, whereas one (*qSFC-Chr09*) was unique to Pop-542 and was consistently identified in all three generations (Table 3). The PV% explained by these QTLs ranged from 3.8 to 15.1%, with genetic effects ranging from 0.1 to 0.8%. Alleles from both Sealand parents increased trait value for all the QTLs

Table 3. Summary of fiber quality quantitative trait loci (QTLs) identified in three generations of Pop542 and Pop883.

Trait	QTL name	Population	LOD†			Additive effect			R^2			+ve‡ allele
			F_2	$F_{2:3}$	$F_{2:4}$	F_2	$F_{2:3}$	$F_{2:4}$	F_2	$F_{2:3}$	$F_{2:4}$	
									PV%§			
Elongation	*qELO-Chr05*	Pop883		3.8			−0.1			2.4		SL883
	qELO-Chr08	Pop542	3.0			0.1			4.2			S7235
	qELO-Chr11	Pop883	4.0	3.7	4.3	−0.2	−0.1	−0.1	2.8	7.4	13.6	SL883
	qELO-Chr21	Pop542		3.8	3.1		−0.2	−0.1		5.8	9.1	SL542
	qELO-Chr23	Pop542	10.6	3.2	3.7	−0.8	−0.1	−0.1	9.5	7.3	5.3	SL542
	qELO-Chr24	Pop542	2.5			−0.1			4.2			SL542
		Pop883		3.1			0.1			6.6		S7235
Fiber fineness	*qMIC-Chr09*	Pop542		3.5	2.6		−0.1	−0.1		10.0	4.7	SL542
	qMIC-Chr11	Pop883	3.9		3.0	−0.4		−0.1	5.8		4.2	SL883
	qMIC-Chr17	Pop883	7.7	9.8	1.7	0.3	0.2	0.1	11.7	7.2	2.4	S7235
	qMIC-Chr24	Pop542	9.6	3.4	7.4	0.3	0.2	0.2	21.3	9.1	9.3	S7235
		Pop883	11.8	2.6	3.5	0.4	0.1	0.2	33.6	6.4	5.4	S7235
Short fiber content	*qSFC-Chr05*	Pop883	3.8			0.2			6.8			S7235
	qSFC-Chr08	Pop883		3.2			−0.1			4.0		SL883
	qSFC-Chr09	Pop542	4.2	4.4	3.6	−1.2	−0.3	−0.2	9.4	8.1	14.1	SL542
	qSFC-Chr17	Pop883		6.5			−0.2			15.1		SL883
	qSFC-Chr24	Pop883	4.6			−0.8			3.8			SL883
Fiber strength (kN m kg^{-1})	*qSTR-Chr08*	Pop542		3.3			−7.2			13.5		SL542
	qSTR-Chr26	Pop542		2.5	2.1		−4.4	−5.5		7.2	8.3	SL542
		Pop883	3.9	3.7		6.4	4.4		6.0	7.6		S7235
	qSTR-Chr16	Pop883	3.4	3.8		−8.8	−3.4		9.4	6.8		SL883
	qSTR-Chr17	Pop883	14.2	5.3	3.7	7.1	6.0	5.9	11.2	10.3	6.4	S7235
	qSTR-Chr24	Pop542	12.5	15.0	15.1	18.9	104.4	91.7	22.0	24.9	18.2	S7235
		Pop883	14.1	15.3	17.4	22.8	117.4	117.6	30.9	40.1	36.4	S7235
Fiber length (mm)	*qFL-Chr09*	Pop542	3.0	3.4		0.8	0.1		4.4	5.1		S7235
	qFL-Chr16	Pop883	4.4		3.5	−0.5		−0.1	8.2		6.5	SL883
	qFL-Chr25	Pop883	5.0	5.7	12.6	−0.8	−0.1	−0.1	15.4	4.2	9.4	SL883
Uniformity index	*qUI-Chr08*	Pop883	3.6	2.6		0.7	0.3		4.0	3.3		S7235
	qUI-Chr26	Pop883		3.7			0.4			7.4		S7235
	qUI-Chr15	Pop883	3.4			−0.4			5.1			SL883
	qUI-Chr17	Pop883		3.2			0.4			6.6		S7235
	qUI-Chr24	Pop542	2.6	3.0	3.2	1.0	0.3	0.1	2.0	2.9	3.9	S7235
		Pop883	7.9	3.1	3.5	1.0	0.4	0.2	7.8	8.5	7.5	S7235

† LOD, logarithm of odds.

‡ ve+, parent contributed the favorable allele.

§ PV%, percentage phenotypic variance.

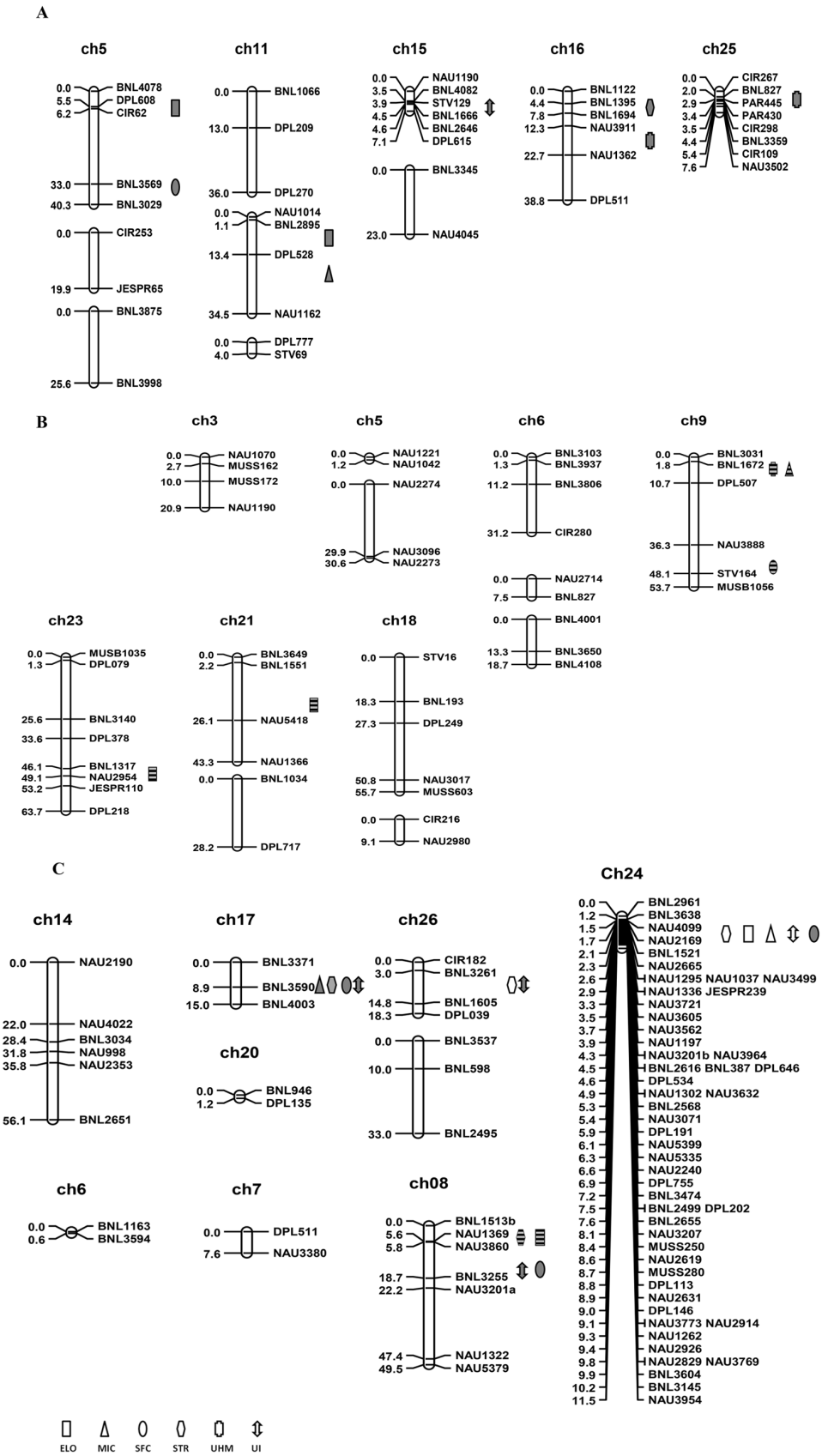

Fig. 3. Linkage groups and position of fiber quality quantitative trait loci (QTLs) detected in the mapping population Pop542 and Pop883. Panel A shows the linkage groups of introgressed segments in SL883, Panel B shows the linkage groups of introgressed segments in SL542, and Panel C shows the linkage groups of polymorphic regions between Sealand lines and S7235. Legends with solid gray represent QTLs detected in Pop883, hashes represent QTLs detected in Pop542, and those with no fill represent common QTLs detected in both Pop542 and Pop883. ELO, elongation; MIC, micronaire (fineness): SFC, short fiber content; STR, strength; UHM, upper half mean length; UI, uniformity index.

except for *qSFC-Chr05*, where S7235 contributed alleles for increasing short fiber content. Alleles for short fiber content from both SL-542 and SL-883 were expected because they had greater short fiber content than S-7235.

Fiber Strength

A total of five QTLs were identified. Two (*qSTR-Chr16* and *qSTR-Chr17*) were unique to Pop-883, whereas one (*qSTR-Chr08*) was unique to Pop-542 (Table 3). Two QTLs (*qSTR-Chr24* and *qSTR-Chr26*) were common between Pop-542 and Pop-883 and were detected in more than one generation of their respective population. The QTL *qSTR-Chr26* was identified in Pop-883; however, it was identified as a putative QTL (LOD $\leq$ 2.5) in Pop-542. The PV% explained by these QTLs ranged from 6 to >40%, with additive effects ranging from 3.4 to 117.6 kN m kg^{-1}. Alleles from S-7235 increased fiber strength at all loci except for *qSTR-Chr08* and *qSTR-Chr16*, where Sealand parents contributed alleles for improved fiber strength.

Fiber Length

A total of three QTLs were identified, two (*qFL-Chr16* and *qFL-Chr25*) unique to Pop-883 and one (*qFL-Chr09*) unique to Pop-542 (Table 3). The QTL *qFL-Chr25* was consistently detected in all three generations of Pop-883. The PV% explained by these QTLs ranged from 4.2 to 9.4%, with additive effects ranging from 0.3 to 0.9 mm. Favorable alleles for *qFL-Chr09* originated from S-7235, whereas Sl-883 alleles improved fiber length at loci *qFL-Chr16* and *qFL-Chr25*. SL-883 had significantly greater fiber length than S-7235, and therefore favorable alleles from SL-883 for fiber length in Pop-883 were expected. Between the parents of Pop-542, S-7235 had significantly greater fiber length, and therefore favorable alleles from S-7235 in Pop-542 were also expected.

Fiber Uniformity

A total of five QTLs were identified. Four (*qUI-Chr08*, *qUI-Chr15*, *qUI-Chr17*, and *qUI-Chr26*) were unique to Pop-883, whereas one (*qUI-Chr24*) was common between the two populations. The QTL *qUI-Chr24* was consistently identified over three generations of both mapping populations (Table 3). The PV% explained by these QTLs ranged from 2.0 to 7.5%, with genetic effects ranging from 0.1 to 1.0%. Favorable alleles that increase the uniformity index for all QTLs were contributed by S-7235, except for *qUI-Chr15*, where the favorable allele originated from SL-883. Favorable alleles were expected to come from S-7235 because it had significantly greater fiber uniformity than both Sealand parents; however, the QTL *qUI-Chr15*, for which SL-883 contributed a favorable allele, may be a transgressive segregant.

DISCUSSION

The use of interspecific introgressive breeding in cotton improvement has been an attractive approach since the beginning of modern upland germplasm development, particularly with the focus on transferring genes from *G. barbadense* to improve the fiber quality (Ware, 1951). Examples of introgressive breeding in upland cotton include two well-known successes in improving fiber quality: fiber strength in Acala cotton (Smith and Cothren, 1999; Zhang et al., 2005), and fiber length in extra-long staple cottons including Sealand and Earlistaple cultivars (Culp and Harrell, 1977). Introgression from *G. barbadense* into the Sealand series was suspected, not only because of their pedigree but also due to their unique fiber quality in lines such as SL-883, which was approaching that of sea island cotton. A genomic survey of the two Sealand cultivars confirmed 22 introgressed chromosome segments ranging in size from 1.2 to 63.7 cM. The introgression events were found to be equally distributed between A_t and D_t subgenomes of tetraploid cotton, and the introgression patterns were unique to each Sealand cultivar. Although chromosome 05 was found to harbor *G. barbadense* introgression in both Sealand lines, the size and location of introgressed regions were different.

The uniqueness in introgression pattern between SL-542 and SL-883 likely accounts for the differences in fiber quality between these two cultivars. The retention of *G. barbadense* chromatin in each Sealand was unique, suggesting that these lines and perhaps others in the Sealand series may have been independently derived during backcrossing. Phenotypic selection pressure for fiber quality traits and yield components applied during backcrossing may have resulted in lines with different but stable introgression of *G. barbadense* alleles. The Germplasm Resources Information Network (GRIN) database (www.ars-grin.gov) indicates that six additional Sealand lines (SL-1, SL-3, SL-7 white flower, SL-7 yellow flower, SL-391, and SL-472) may also have been independently derived, and thus the genomic composition of each of these lines may be unique. Therefore, the Sealand lines together as a group may serve as reservoir of novel fiber quality alleles of *G. barbadense* origin. Furthermore, the phenotypic observations at GRIN show that the remaining Sealand lines also have unique fiber quality and yield traits, and therefore it may be worthwhile to explore these lines for novel yield and fiber quality alleles.

Sealand lines contributed favorable alleles for 17 of the 28 fiber quality QTLs identified in this study, 12 of which were detected on the introgressed segments. Three introgressed segments in SL-542 contributed favorable alleles for five QTLs (*qELO-Chr21*, *qELO-Chr23*, *qMIC-Chr09*, *qSFC-Chr09*, and *qFl-Chr09*), whereas five introgressed segments in SL-883 contributed favorable alleles for seven QTLs (*qELO-Chr05*, *qELO-Chr11*, *qMIC-Chr11*,

qSTR-Chr16, *qFL-Chr16*, *qFL-Chr25*, and *qUI-Chr15*). A single introgression on chromosome 16 in SL-883 harbored favorable alleles for both fiber length and fiber strength. A noteworthy observation is that all chromosomes registering an introgression event in SL-883 harbored one or more fiber quality QTLs. In contrast, only four of the seven chromosomes registering introgression event in SL-542 harbored fiber quality QTLs. A possible explanation for this observation is that perhaps the retention of these segments may have been due to the selection of other agronomic traits such as yield components. The GRIN observation shows that SL-542 has better lint percentage than SL-883 and other Sealand lines. Alternatively, some of these *G. barbadense* segments could represent random introgression with no improvement value.

Many QTLs were identified in the same marker interval; for example, chromosomes 16 and 24 carried multiple fiber quality QTLs. The correlation observed among fiber quality traits may be the direct result of colocalization of fiber quality QTLs. Colocalization of QTLs is observed in many crops (Saliba-Colombani et al., 2001; Lexer et al., 2003; Yan et al., 2009; Acuña-Galindo et al., 2015) including cotton (Lacape et al., 2010; Shen et al., 2011; Zhang et al., 2011; Liu et al., 2015), where QTLs with both positive and negative genetic effects were detected at the same chromosomal position. Another noteworthy observation is that 16 (or 57%) of the fiber quality QTLs were identified on D_t subgenome. Although the D subgenome progenitor did not produce spinnable fibers, it has loci that influence the quality of the fiber produced in allotetraploid cottons, which indicates that the polyploidization of *Gossypium* has given rise to novel variation for fiber quality traits (Jiang et al., 1998; Wendel, 2000). Similar results were previously reported in cotton (Jiang et al., 1998; Paterson et al., 2003; Mei et al., 2004; Chee et al., 2005b; Rong et al., 2007; Yu et al., 2013; Xu et al., 2015), where the genetic control of fiber quality by the D_t subgenome was significantly greater than that of A_t subgenome.

The validation of the QTLs detected in this study is an essential step toward using them for marker-assisted selection to improve fiber quality (Chee and Campbell, 2009). Of the 28 QTL identified in this study, eight (29%) were detected in the F_2, $F_{2:3}$, and $F_{2:4}$ generations, indicating that these QTLs are stably expressed across environment and generation. In addition, two consistent QTLs, *qELO-Chr21* and *qMIC-Chr09*, which were mapped to the introgressed segment in the SL-542 line, were detected in the $F_{2:3}$ and $F_{2:4}$ generations. Another obstacle in implementing marker-assisted selection to improve fiber quality is the incomplete understanding of the QTL position, as well as its predictive phenotypic effect in different genetic backgrounds (Chee and Campbell, 2009). Since the two Sealand parents are genetically distinct, Pop-542 and Pop-883 provide not only information on detection but also validation for common QTLs contributed by the S-7235 parent. Therefore, the four common QTLs *qMIC-Chr24*, *qSTR-Chr26*, *qSTR-Chr24*, and *qUI-Chr24* that were contributed by the S-7235 parent are likely to be authentic. In addition, because of the population design, we detected significant interactions between these common QTLs and the genetic backgrounds. For example, the S-7235 allele for *qMIC-Chr24* accounted for greater genetic variation when present in an SL-542 background, whereas alleles for the QTLs *qUI-Chr24* and *qSTR-Chr24* explained a larger PV% when present in an SL-883 genetic background (Kumar et al., 2012). Similarly, the QTL *qSTR-Chr26* had opposing effects in different genetic backgrounds. For this QTL, the SL-542 allele increase fiber strength in Pop542; however, the same allele when present in Pop883 resulted in lower fiber strength. Such epistatic interactions between QTLs and genetic backgrounds have been observed in other interspecific populations with alien chromosome segments in cotton (Chee et al., 2005a, 2005b; Zhang et al., 2011) and other crop species (Sebolt et al., 2000; Liao et al., 2001; Lecomte et al., 2004; Blanc et al., 2006; Li et al., 2009).

Both SL-542 and SL-883 contributed positive QTL alleles for important fiber quality traits. These results support previous speculation that many of the *G. barbadense* chromosomal segments in SL-542 and SL-883 were retained as a result of phenotypic selection pressure imposed during backcrossing. For example, of the six fiber elongation QTLs identified, favorable alleles for four QTLs originated from the introgressed *G. barbadense* chromatin. Of the two Sealand parents, SL-542 has significantly greater fiber elongation, which could be attributed to the QTLs present in chromosomes 21 and 23. Interestingly, fiber elongation was not a selection criterion during the development of the Sealand lines (May, 1999). However, it is possible that the improvement in fiber elongation of SL-542 resulted from its correlation with other fiber quality traits such as fiber strength and fineness, which were emphasized in cotton breeding during that time (May, 1999). Other examples of favorable alleles contributed by the Sealand parents include two QTLs for micronaire and three QTLs for short fiber content. Micronaire values are indicators of both fiber maturity and fiber fineness, with higher values (>4.5) indicating more mature cotton fibers and lower values (<3.5) indicating immature fibers. Lower micronaire fibers are finer in texture and are sought by the textile mills; however, when lower fineness values predominate due to immature fiber, this can cause neps and dye defects (Draye et al., 2005). Finally, two fiber length QTLs were contributed by the SL-883 parent, and the consistent QTL *qFL-Chr25* was detected at a very high confidence level (13 LOD) in the $F_{2:4}$ generation. Longer fibers require less twist in the roving process during ring spinning and thus are required for the production of finer yarn (May, 1999).

Correlations among fiber quality traits could occur either due to linkage or pleiotropy. In the present study, we found a positive allele for fiber length and fiber strength on the introgression segment on chromosome 16 of SL-883 and a positive allele for fiber strength and uniformity index on chromosome 24 of S-7235, suggesting that the correlation between these traits could be in part due to colocalization of QTLs. Colocalization of QTLs has been demonstrated in several crops including *Brassica* (Yan et al., 2009), sunflower (*Helianthus paradoxus* Heiser; Lexer et al., 2003), and tomato (*Solanum lycopersicum* L.; Saliba-Colombani et al., 2001). In cotton, QTLs for fiber quality have been observed to be colocalized and confined in a QTL rich region across the cotton genome (Saranga et al., 2001; Chee et al., 2005b; Lacape et al., 2005; Rong et al., 2007; Lacape et al., 2010; Shen et al., 2011). Colocalization of QTLs with desirable effects could simplify their manipulation but becomes problematic when the QTLs are of opposite effects or are linked to poor agronomic traits such as low lint yield.

In conclusion, the favorable alleles on stably introgressed segments in the two Sealand parents are an important reservoir for improving fiber quality traits in upland cotton. Many of the QTLs reported herein displayed consistency over generations with moderate to high heritability. The DNA markers delineating their precise genetic locations would allow these QTLs to be integrated into elite cotton germplasm via marker-assisted selection to improve fiber quality.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Standards

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

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