agriculture

MDPI

Article

QTL Mapping for Fiber Quality Based on Introgression Lines Population from *G. hirsutum* × *G. tomentosum*

Xinyi Chang [1,†], Chunping Guo [1,†], Zhenyuan Pan [1], Yuanlong Wu [1], Chao Shen [2], Lei Chao [1], Guangling Shui [1], Chunyuan You [3,4], Jianwei Xu [1,*], Zhongxu Lin [1,3,*] and Xinhui Nie [1,*]

1 Key Laboratory of Oasis Ecology Agricultural of Xinjiang Production and Construction Corps, Agricultural College, Shihezi University, Shihezi 832003, China
2 College of Biological and Food Engineering, Guangdong University of Petrochemical Technology, Maoming 525000, China
3 National Key Laboratory of Crop Genetic Improvement, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
4 Cotton Research Institute of the Shihezi Academy of Agriculture Science, Shihezi 832011, China
* Correspondence: jwchxjw@shzu.edu.cn (J.X.); linzhongxu@mail.hzau.edu.cn (Z.L.); xjnxh2004130@126.com (X.N.)
† These authors have contributed equally to this work and share first authorship.

Abstract: As one of the most widely cultivated cotton species in China, upland cotton has moderate fiber quality and wide applicability, but its genetic basis is relatively narrow. To expand genetic diversity and improve fiber quality, in this study an introgression population (BC_5S_5) containing 107 lines was constructed by using *G. hirsutum acc.* 4105 as the recurrent parent and *G. tomentosum* as the donor parent. Using the specific-locus amplified fragment sequencing (SLAF-seq) strategy, 3157 high-throughput single nucleotide polymorphism (SNP) markers were obtained. Linkage analysis showed that a total of ninety-one QTLs related to fiber quality traits were detected in three environments, and the phenotypic variance explained (PVE) rates were 4.53–20.92%. Forty-six QTL (50.55%) synergistic genes were derived from *G. tomentosum*. Among them, *qFS-A02-1* and *qSCI-A02-1* were stably detected with a PVE of 9.8–16.71% and 14.78–20.92%, respectively. Within the candidate interval, *Ghir_A02G012730*, *Ghir_A02G012790* and *Ghir_A02G012830* were found to be possibly involved in cellulose and cell wall biosynthesis, with a relatively high expression during fiber development, 20 DPA and 25 DPA, which suggested that these three genes may be involved in the regulation of fiber strength traits, but their functions need further validation to determine the regulatory mechanism. Our research lays the foundation of fiber quality related to basic genetic research and breeding in cotton.

Keywords: cotton; fiber quality; introgression line population; QTL mapping; linkage analysis

Citation: Chang, X.; Guo, C.; Pan, Z.; Wu, Y.; Shen, C.; Chao, L.; Shui, G.; You, C.; Xu, J.; Lin, Z.; et al. QTL Mapping for Fiber Quality Based on Introgression Lines Population from *G. hirsutum* × *G. tomentosum*. *Agriculture* **2023**, *13*, 579. https://doi.org/10.3390/agriculture13030579

Academic Editors: Rakesh Singh and Wen-Chi Chang

Received: 7 December 2022
Revised: 5 February 2023
Accepted: 20 February 2023
Published: 27 February 2023

1. Introduction

Cotton, as one of the important economic crops in China, has an important role in the national economy because its fiber is the primary raw material in the textile industry [1]. At present, due to its high yield and wide adaptation, upland cotton (*Gossypium hirsutum* L.) accounts for more than 95% of global cotton production. However, it is difficult to meet the market demand due to the medium quality of fiber [2]. The global demand for cotton production has still been increasing in recent years. Due to the fierce competition of synthetic fiber and other fiber crops, and the increase in production cost, China still pursues import for high-quality cotton fiber from abroad. This suggest that the improvement of the fiber quality in high-yield cotton is very important [3].

Fiber quality is a complex trait controlled by a magnitude of QTLs [4]. With the rapid development of molecular marker technology, molecular markers have gone through the development of the first generation (represented by RFLP), the second generation

(represented by SSR) and the third generation (represented by SNP) [5]. Linkage analysis involves using the linkage recombination between functional genes and markers to compare whether there are significant differences in the mean values of different marker genotypes so as to achieve the mapping of functional genes [6]. Until now, draft genomes of several cotton species have been released, including *Gossypium raimondii* [7], *Gossypium arboreum* [8], *Gossypium hirsutum* [9] and *Gossypium barbadense* [10]. This greatly promoted the progress of QTL mapping for cotton agronomic traits and functional gene mining and made an important contribution to cotton molecular breeding.

Wild cotton species have gained excellent characteristics and adaptation mechanisms to resist various adverse factors under long-term natural selection. At present, nearly twenty kinds of wild cotton species (*G. mustelinum*, *G. darwinii* and *G. tomentosum*, etc.) have been used for genetic breeding and improvement [11]. Using *G. mustelinum* (donor parent) to develop a chromosome segment substitution lines (CSSLs) population consisting of seventy-one lines, a total of twenty-nine QTLs related to six fiber quality traits were detected [12]. The association mapping of 105 introgression lines obtained by the interspecific cross of *G. darwinii* with four pieces of upland cotton showed that forty Simple Sequence Repeats (SSRs) were correlated with five fiber quality traits ($p < 0.05$) [11]. *G. tomentosum* is one of the allotetraploid wild cotton species which has the advantages of drought resistance, disease and insect resistance, high fiber strength, hairy and no nectaries [13]. Seventeen BC_3F_1 lines were bred by crossing two upland cotton varieties (CA3084 and CA3093) with *G. tomentosum*. A total of twenty-eight QTLs were detected for QTL mapping of fiber quality traits by BC_3F_2 obtained by self-crossing [14]. Thirty QTLs related to five fiber quality traits were detected by using 107 introgression lines, which were developed with an interspecific cross using *G. tomentosum* as the donor parent and *G. hirsutum acc*. 4105 as the recurrent parent, and mainly distributed on chromosomes A01, A09, A13, D02 and D10, respectively [15]. A total of ninety-two QTLs related to five fiber quality traits were detected by QTL mapping for fiber quality traits from the chromosome segment substitution lines of *G. tomentosum*. Additionally, these QTLs had a range of 2.0–12.6% PVE. Among them, there are eighty QTL-favorable alleles from *G. tomentosum* [16].

In this study, the upland cotton variety (*G. hirsutum acc*. 4105) was used as the recurrent parent and *G. tomentosum* as the donor parent to construct BC_5S_5 populations (Figure S1). QTL mapping of cotton fiber quality traits was detected based on phenotypic values in multi-environments, which laid a foundation for using favorable alleles of important agronomic traits to improve fiber quality in upland cotton.

2. Materials and Methods

2.1. Plant Materials and Field Experiments

In this study, *G. tomentosum* was used as the donor parent, and *G. hirsutum acc.* 4105 was used as the recurrent parent to construct a BC_5S_5 population with 107 lines (Figure S1) [15]. The population was planted in Manasi County in the north of Xinjiang in 2018 (E1), Korla in the south of Xinjiang in 2018 (E2) and Manasi County in the north of Xinjiang in 2019 (E3). In all environments, the field experiment was designed by means of completely randomized block design. The planting pattern was four rows per film, and the row spacing (28 + 50 + 28) + 55 cm, plant spacing 9.5 cm, row length 5 m and 2 repetitions. All environments throughout the growth period adopt a unified field management model.

2.2. Collection of Fiber Quality Traits

Ten naturally opened bolls from the middle of the BC_5S_5 population were gathered in E1, E2 and E3 from mid to late September to October each year. The collected bolls were sent to the Cotton Research Institute of Xinjiang Shihezi Academy of Agricultural Sciences for testing. The fiber quality traits were tested by the HVI-1000 Automatic Fiber Measurement System (USTER® HVI 1000, Uster Technologies, Uster, Switzerland) at twenty degrees Celsius and relative humidity of sixty-five percent. Nine fiber quality traits were checked, including fiber length (FL), fiber strength (FS), maturity of cotton fiber (MCF),

micronaire value (MIC), short fiber content (SFC), fiber uniformity (FU), fiber elongation (FE), spinning consistency index (SCI) and the water content (TWC).

2.3. Statistics of Phenotypic Data

The statistical software SPSS (IBM SPSS Statistics, version 24, Armonk, NY, USA https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-24) (accessed on 22 April 2021) [17] was used to analyze variance and basic statistical analysis of the phenotypic data of fiber quality traits, such as average, standard deviation, coefficient of variation, kurtosis, skewness and so on. The correlation analysis of fiber quality traits was carried out by using the "corrplot" package in R program (https://cran.r-project.org/web/packages/corrplot/index.html) (accessed on 23 April 2021) [18]. The heritability (H^2) analysis was carried out by using the "lme4" package in R program (https://cran.r-project.org/web/packages/lme4/index.html) (accessed on 23 April 2021) [19].

2.4. Identification of SNP Markers

SNP markers were obtained by SLAF-sequences analysis and SNP identification as described by Keerio et al. [15]. The young and fresh leaves of parents and each introgression line (IL) were collected, frozen with liquid nitrogen and stored in the refrigerator at −70 °C. After the DNA was extracted, agarose gel electrophoresis (1%) was performed to meet the requirements of database construction, and then SLAF-seq database construction, sequencing, tag analysis and SNP identification were carried out [20,21]. The SLAF-sequences of *G. tomentosum* were from Shen et al. [20]. The sequencing depth of parents is more than 20 times, and the sequencing depth of offspring is more than 5 times. Raw reads were first used to filter out low-quality reads (quality score < 20 e), the remaining reads were sorted to each progeny according to duplex barcode sequences. Then, each of the high-quality reads was trimmed off 5-bp terminal position. The reference genome TM-1 sequence was downloaded from the CottonGen database (https://www.cottongen.org) (accessed on 15 June 2020). High-quality reads were mapped to the genome of *G. hirsutum* TM-1 using Burrows-Wheeler-Aligner (BWA, version 0.7.10) software [22]. Sequences that map to the same position with more than 95% identity are defined as a SLAF locus. SNP loci in each SLAF locus were then detected between parents using the Genome Analysis Toolkit (GATK) software. SNPs were filtered with the criteria that the minimum read depth was less than 10, and the average base quality was less than 30. A total of 25,659 SNP markers were detected in the parents, of which 20,370 SNP markers were located on chromosomes. Finally, 3157 SNP markers were obtained by further comparison with the SNP in each IL. The genome sequences of ILs developed from SLAF-seq are available at NCBI Sequence Read Archive database with BioProject accession number PRJNA421265 and PRJNA316549.

2.5. QTL Mapping

The QTL IciMapping version 4.2 software (https://isbreeding.caas.cn/rj/index.htm) (accessed on 10 March 2022) was used to construct the genetic map based on the 3157 SNPs data, and the JoinMap 4.0 software was used to visualize the genetic map. To detect the QTL related to fiber quality traits, the CSL function of QTL IciMapping 4.2 software [23] was used to detect fiber quality traits QTL based on the stepwise regression additive effect model (RSTER-LRT-ADD). Genotyping of SNP according to QTL IciMapping 4.2 software requirements was performed [24]. We marked the same genotype as *G. hirsutum acc.* 4105 as "0" and the same genotype as *G. tomentosum* as "2" for QTL mapping. An LOD threshold of 3.0 was considered to define a significant additive QTL. If the same QTL is detected in more than two environments, it will be defined as a stable QTL. The R program "LinkageMapView" package (https://cran.r-project.org/web/packages/LinkageMapView/) (accessed on 10 March 2022) [25] was used to visualize the QTL on the genetic map. Naming method of QTL: q + trait abbreviation + "-" + chromosome number + QTL number [26]. Stable QTLS are those that can be detected simultaneously in two or more environments [27].

2.6. RNA Extraction and Gene Expression Validation

The experimental material is the cotton variety TM-1. Cotton fibers were collected at different developmental stages (0, 5, 10, 15, 20 and 25 DPA) and stored in −80 °C ultra-low-temperature refrigerator after snap-freezing using liquid nitrogen. Total RNA was extracted using the EASYspin Plus Plant RNA Kit (Aidlab Biotech, Beijing, China), and the RNA concentration and purity were determined by the NanoDrop spectrophotometry and agarose gel electrophoresis, respectively. The RNA reverse transcribes into cDNA using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit. To verify the potential functions of candidate genes in cotton fiber development, primer design was performed using the online site Primer3Plus (https://www.primer3plus.com/index.html) (accessed on 2 May 2022), the primers for qRT-PCR analysis are listed in Table S1. The qRT-PCR method was used to verify the expression patterns of genes at different periods of fiber development [28] by using the PerfectStart Green qPCR SuperMix Kit for validation. The gene relative expression level was calculated by the $2^{-\Delta\Delta Ct}$ method [29]. Three independent replicates were performed for each sample. The *GhUBQ7* (accession number: DQ116441) gene was used as a reference gene.

3. Results

3.1. Phenotypic Statistical Analysis

To uncover the excellent allelic variation of fiber quality from *G. tomentosum*, a BC_5S_5 population of 107 introgression lines was constructed, and the fiber quality traits of the BC_5S_5 population and its recurrent parents (*G. hirsutum acc.* 4105) were investigated in 2018 and 2019. All the traits exhibited normal distribution based on absolute kurtosis and skewness value being less than 1, with skewness ranging from −0.18 to 0.51 and kurtosis ranging from −0.86 to 5.8 (Table 1, Figure S2). The coefficient of variation (CV) varied from 1.18% (MCF) to 17.79% (FE), with an average CV of 7.68%. The heritability (H^2) ranged from 17.95% (SFC) to 74.29% (FE) (Table 1). Under the three environments, traits such as FL, FS and SCI all showed a large degree of variability and the phenomenon of transgressive segregation [30], indicating that these traits were quantitative traits controlled by polygenes and could be mapped by QTLs. The phenotypic trends of eight traits available in three environments are shown in Figure 1, the phenotype data of FL, FS, MIC, SCI and FU were more stable than other traits among the three environments, while MCF, FE, TWC and SFC were more easily affected by the environment, among which TWC had the worst stability and was greatly affected by the environment.

Table 1. Descriptive statistics of values for fiber quality traits under three environments.

Trait	Environment	4105 Average	ILs Average	Max.	Min.	SD	CV (%)	Skewness	Kurtosis	H^2 (%)
FL (mm)	E1	29.21	28.49	31.74	25.76	1.33	4.66	0.13	−0.62	61.48
	E2	29.03	28.56	32.89	24.66	1.80	6.29	0.31	−0.12	
	E3	27.69	28.73	31.34	26.20	1.10	3.83	0.23	−0.39	
FS ($cN.tex^{-1}$)	E1	30.20	30.56	36.06	24.4	2.21	7.24	0.04	0.00	61.4
	E2	28.25	29.08	33.94	24.55	2.04	7.02	0.06	−0.31	
	E3	29.73	29.77	34.34	25.69	1.96	6.60	0.14	−0.33	
MIC	E1	4.50	4.32	5.14	3.25	0.37	8.69	0.00	−0.18	55.56
	E2	3.34	4.15	5.16	2.96	0.49	11.82	−0.14	−0.56	
	E3	4.75	4.25	5.19	3.26	0.33	7.68	−0.18	0.58	
MCF (%)	E1	86.93	86.77	89.19	84.16	0.01	1.18	0.00	−0.22	54.3
	E2	84.27	86.27	89.81	83.27	0.01	1.68	0.08	−0.59	
	E3	86.96	85.59	88.64	83.05	0.01	1.19	0.19	0.53	
FU (%)	E1	85.61	84.67	87.38	82.59	1.03	1.22	0.36	−0.21	22.62
	E2	87.72	84.03	87.66	81.07	1.46	1.73	0.21	−0.58	
	E3	85.67	84.93	87.14	82.42	1.13	1.33	−0.13	−0.44	

Table 1. *Cont.*

Trait	Environment	4105 Average	ILs Average	Max.	Min.	SD	CV (%)	Skewness	Kurtosis	H^2 (%)
SFC (%)	E1	8.51	9.56	12.79	6.36	1.56	16.37	0.13	−0.86	17.95
	E2	8.24	11.88	17.53	7.38	2.26	19.06	0.44	−0.23	
	E3	7.12	9.31	12.08	6.50	1.16	12.43	0.02	−0.28	
FE (%)	E1	5.95	5.57	7.99	3.84	0.97	17.35	0.51	−0.26	74.29
	E2	5.62	5.55	7.88	3.57	0.99	17.79	0.17	−0.49	
	E3	7.17	6.90	9.18	4.66	0.91	13.23	0.02	−0.3	
SCI	E1	142.82	140.33	163.11	117.5	11.05	7.87	0.17	−0.58	57.08
	E2	156.28	134.81	164.35	109.08	13.68	10.15	0.00	−0.68	
	E3	135.05	138.19	163.43	112.91	10.71	7.75	−0.08	−0.38	
TWC (%)	E1	6.22	6.54	7.28	5.96	0.31	4.74	0.48	−0.15	40
	E2	6.11	6.20	7.04	5.72	0.26	4.25	0.41	−0.08	
	E3	6.71	7.67	8.40	7.07	0.32	4.13	0.22	−0.52	

Note: E1 for Manasi (2018); E2 for Korla (2018); E3 for Manasi (2019); FL: fiber length; FS: fiber strength; MCF: maturity of cotton fiber; MIC: micronaire value; SFC: short fiber content; FU: fiber uniformity; FE: fiber elongation; SCI: spinning consistency index; TWC: the water content.

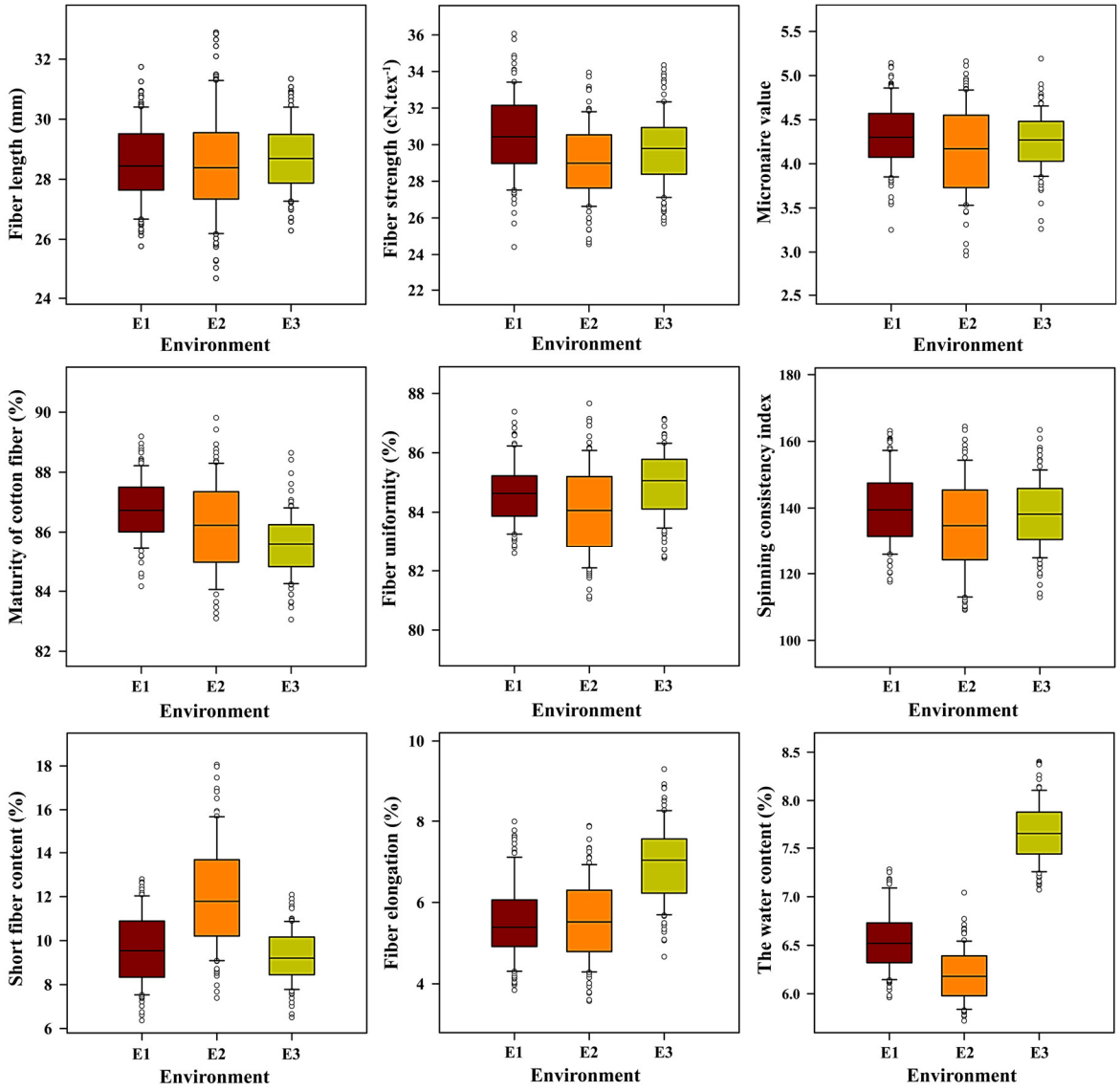

Figure 1. Statistics of nine fiber quality traits under three environments: E1 (Manasi in 2018), E2 (Korla in 2018), E3 (Manasi in 2019).

3.2. Phenotypic Correlation Analysis

Further, the correlation analysis of fiber-quality-related traits was carried out by using the "corrplot" package in R (Figure 2) and found that FL was significantly positively correlated with FS, FU and SCI, while it was negatively correlated with MIC, MCF and SFC. FS was positively correlated with MCF, FU and SCI, and negatively correlated with SFC. However, MIC had no significant correlation with other traits except that there was a significant positive correlation with MCF and a significant negative correlation with SCI and FL. MCF was significantly negatively correlated with FL, FE, TWC and SCI. FU was negatively correlated with SFC and positively correlated with FE, TWC and SCI. Among them, SFC was not significantly correlated with MIC and MCF, but was negatively correlated with other fiber quality traits. There was a significant positive correlation between FE and TWC. Conversely, there was no significant correlation between TWC and SCI.

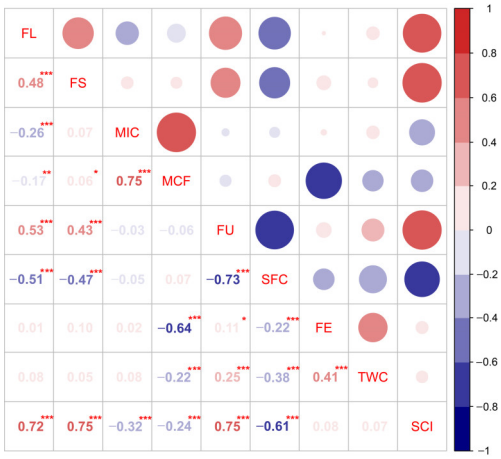

Figure 2. Pearson's correlation coefficient among fiber quality traits. * Indicates significant difference at the 0.05 probability level; ** indicates significant difference at the 0.01 probability level; *** indicates significant difference at the 0.001 probability level. FL: fiber length; FS: fiber strength; MCF: maturity of cotton fiber; MIC: micronaire value; SFC: short fiber content; FU: fiber uniformity; FE: fiber elongation; SCI: spinning consistency index; TWC: the water content.

3.3. Phenotypic Variance Analysis

Moreover, variance analysis of fiber quality traits in three environments was carried out. As shown in Table 2, except for SFC and TWC, other traits had genotypic differences with extreme significance. Furthermore, all fiber quality traits from different environments showed significant variation. In the interaction between genotype and environment, except for SFC, there were significant differences in other traits, and there were extremely significant differences in FL, FE and MIC. Therefore, fiber quality traits are easily affected by genes, different years and different planting environments.

Table 2. Analysis of variance (ANOVA) among fiber quality traits in different environments.

Source	Genotype (*df* = 106)	Environment (*df* = 2)	Genotype × Environment (*df* = 206)	Error (*df* = 103)
FL	5.258 ***	4.367 *	2.040 **	0.823
FS	3.438 ***	15.849 ***	1.388 *	2.772
MIC	4.261 ***	10.946 ***	1.763 ***	0.08
MCF	3.456 ***	60.282 ***	1.380 *	0.873
FU	1.959 ***	24.028 ***	1.498 *	1.227
SFC	0.268	3.477 *	0.277	56.097
FE	3.107 ***	131.828 ***	0.803 **	0.771

Table 2. *Cont.*

Source	Genotype (*df* = 106)	Environment (*df* = 2)	Genotype × Environment (*df* = 206)	Error (*df* = 103)
SCI	2.367 ***	3.802 *	1.038 *	142.465
TWC	1.05	551.562 ***	0.639 *	0.168

Note: * Indicates significant difference at the 0.05 probability level; ** indicates significant difference at the 0.01 probability level; *** indicates significant difference at the 0.001 probability level. FL: fiber length; FS: fiber strength; MCF: maturity of cotton fiber; MIC: micronaire value; SFC: short fiber content; FU: fiber uniformity; FE: fiber elongation; SCI: spinning consistency index; TWC: the water content.

3.4. Genetic Map Construction

To construct the genetic map of the BC_5S_5 population, a total of 3157 SNP polymorphic markers were found in the introduction lines of the BC_5S_5 population, which were unevenly distributed on each chromosome, by SLAF-seq sequencing. The SNP markers on each chromosome range from 24 (D03) to 535 (A13) (Table 3; Figure S3). The genetic linkage map data were visualized by using JoinMap 4.0 software [31]. In total, 3157 markers were assigned to 26 chromosomes, and the total physical distance of the introduced segments was 665 Mb (Table 3; Figure S4). The At sub-genome and Dt sub-genome were 403 Mb (the average coverage rate is 35.44%) and 262 Mb (the average coverage rate is 33.64%), respectively. The highest percentage of genome coverage was 77.54% on chromosome A13, and the smallest was 19.28% on chromosome D03. For each line, it was observed that the SNP markers showed an uneven distribution, among which the IL_64 line contained the most markers (924 SNPs); while the IL_80 line had only 196 SNPs (Figure 3). Genotyping of 107 strains of the BC_5S_5 population revealed that there were identical imported fragments among different families on chromosomes A06 and A13 of the At subgenome and chromosomes D06, D09 and D10 of the Dt subgenome. In particular, chromosomes were most abundant at A13 and D06 (Figure S5).

Table 3. Genome coverage of introgressed chromosome segments in the BC_5S_5 population.

Chromosome	Size of Physical Distance (Mb)	Length of Introgressed Segments in Genome (Mb)	Number of SNP Markers	Average Marker Distance (Mb)	Max Interval (Mb)	Percentage of Genome Coverage (%)
A01	99.88	38	317	0.31	16.91	38.04
A02	83.45	34	99	0.84	28.19	40.74
A03	100.26	22	65	1.49	17.33	21.94
A04	62.91	13	30	2.12	23.27	20.66
A05	82.05	29	81	1.11	26.61	35.35
A06	103.17	24	82	1.25	46.03	23.26
A07	78.25	24	95	0.78	29.57	30.67
A08	103.63	39	108	0.94	14.27	37.64
A09	75.00	30	158	0.45	10.99	40.00
A10	100.87	29	83	1.21	21.00	28.75
A11	93.32	19	55	1.71	36.06	20.36
A12	87.48	40	108	0.81	8.56	45.72
A13	79.96	62	535	0.14	4.76	77.54
At-total	1150.23	403	1816			
Average	88.48	31.00	139.69	1.01	23.23	35.44
D01	61.46	15	39	1.55	22.78	24.41
D02	67.28	34	245	0.27	8.99	50.53
D03	46.69	9	24	1.86	22.18	19.28
D04	51.45	22	79	0.64	17.27	42.76
D05	61.93	21	65	0.84	9.97	33.91
D06	64.29	38	384	0.16	5.40	59.10
D07	55.31	32	178	0.29	4.99	57.85
D08	65.89	14	58	1.14	35.71	21.25
D09	51.00	12	41	1.23	31.77	23.53
D10	63.37	19	101	0.61	14.97	29.98
D11	66.09	13	42	1.60	34.50	19.67
D12	59.11	12	30	1.82	26.46	20.30
D13	60.53	21	55	1.07	11.46	34.69
Dt-total	774.40	262	1341			
Average	59.57	20.15	103.15	1.01	18.96	33.64
Total	1924.63	665	3157			

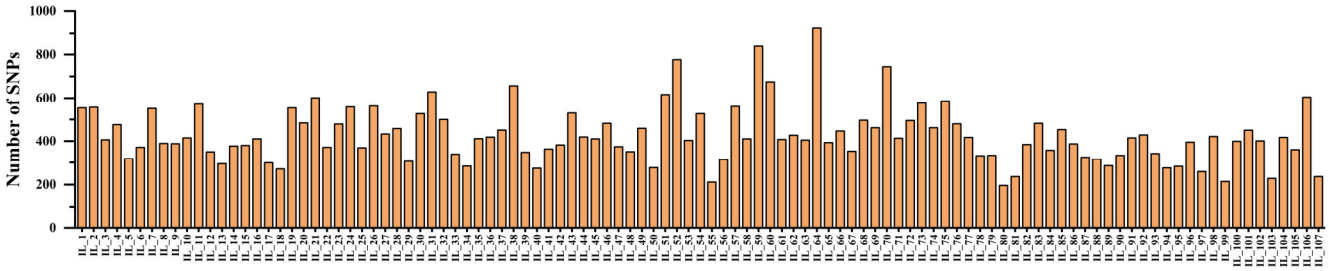

Figure 3. Distribution of SNPs in 107 ILs.

3.5. QTL Mapping of Fiber Quality Traits

In order to map the QTL of fiber quality traits, the CSL function of QTL IciMapping version 4.2 (https://isbreeding.caas.cn/rj/index.htm) (accessed on 10 March 2022) software was used to detect fiber quality traits QTL based on the stepwise regression additive effect model (RSTER-LRT-ADD) (Table 4; Figure 4). A total of 91 QTLs for fiber quality traits were detected on 25 chromosomes. The specific distribution is shown in Figure S6. Thirty-two, twenty and thirty-nine QTLs were detected in E1, E2 and E3 environments, respectively (Table 4). Among the 91 QTLs, 54 QTLs (59.34%) were identified in the At sub-genome, and 37 QTLs (40.66%) were in the Dt sub-genome (Figure S6). The logarithm of odds (LOD), position, PVE, and additive effects of QTLs are presented in Table 4. The PVE of nine fiber quality traits ranged from 4.53% to 20.92%, with an average of 10.54%.

Table 4. QTLs for fiber quality traits detected in ILs populations under three environments.

Trait	Environment	Chromosome	Position (Mb)	QTL	LOD	PVE (%)	Add	Source of Favorable Alleles
FL	E1	A01	33.83	*qFL-A01-1*	6.32	10.24	−0.57	4015
	E3	A03	5.28	*qFL-A03-1*	4.02	6.04	−0.73	4015
	E3	A05	4.58	*qFL-A05-1*	3.38	5.11	−0.34	4015
	E3	A05	60.15	*qFL-A05-2*	5.05	7.76	0.63	*G. tomentosum*
	E3	A06	32.34	*qFL-A06-1*	3.41	5.06	0.57	*G. tomentosum*
	E1	A08	83.82	*qFL-A08-1*	7.19	11.89	−0.82	4015
	E3	A08	7.97	*qFL-A08-2*	3.22	4.74	−0.41	4015
	E3	A10	20.72	*qFL-A10-1*	8.56	14.23	0.52	*G. tomentosum*
	E1	D02	3.37	*qFL-D02-1*	9.45	16.46	−0.61	4105
	E2	D02	1.50	*qFL-D02-2*	4.48	14.91	−0.72	4105
	E3	D02	21.93	*qFL-D02-3*	8.95	15.02	0.50	*G. tomentosum*
	E1	D04	37.79	*qFL-D04-1*	6.59	10.75	0.78	*G. tomentosum*
	E1	D06	52.24	*qFL-D06-1*	4.05	6.32	−0.42	4015
	E3	D08	28.80	*qFL-D08-1*	4.65	7.08	1.74	*G. tomentosum*
	E3	D08	33.59	*qFL-D08-2*	3.08	4.53	0.99	*G. tomentosum*
FS	E1	A02	81.75	*qFS-A02-1*	5.51	9.80	1.04	*G. tomentosum*
	E2	A02	81.75	*qFS-A02-2*	7.59	16.71	1.21	*G. tomentosum*
	E3	A03	95.37	*qFS-A03-1*	3.02	7.23	0.57	*G. tomentosum*
	E2	A10	40.14	*qFS-A10-1*	4.89	10.10	1.65	*G. tomentosum*
	E1	A13	26.20	*qFS-A13-1*	8.70	16.67	−4.09	4015
	E2	A13	78.98	*qFS-A13-2*	3.84	7.74	−0.76	4015
	E1	D07	26.27	*qFS-D07-1*	3.75	6.41	−1.05	4015
	E1	D08	30.65	*qFS-D08-1*	3.33	5.64	0.72	*G. tomentosum*
	E2	D08	30.85	*qFS-D08-2*	5.26	10.97	4.10	*G. tomentosum*
	E3	D08	32.26	*qFS-D08-3*	3.40	8.20	−1.31	4015
	E3	D08	42.32	*qFS-D08-4*	6.11	15.66	−1.14	4015
	E1	D09	46.21	*qFS-D09-1*	7.98	15.02	1.81	*G. tomentosum*
	E3	D11	16.80	*qFS-D11-1*	3.45	8.33	1.05	*G. tomentosum*
MIC	E1	A03	90.56	*qMIC-A03-1*	4.32	10.08	0.26	*G. tomentosum*
	E1	A04	59.45	*qMIC-A04-1*	5.12	12.17	−0.22	4015
	E1	A07	50.02	*qMIC-A07-1*	5.13	12.19	−0.33	4015
	E3	A08	69.93	*qMIC-A08-1*	3.37	11.38	−0.11	4015
	E3	D01	42.61	*qMIC-D01-1*	3.71	12.62	−0.14	4105

Table 4. *Cont.*

Trait	Environment	Chromosome	Position (Mb)	QTL	LOD	PVE (%)	Add	Source of Favorable Alleles
MCF	E2	A02	41.92	*qMCF-A02-1*	8.78	16.17	0.03	*G. tomentosum*
	E1	A03	89.63	*qMCF-A03-1*	3.64	11.25	0.01	*G. tomentosum*
	E3	A05	35.08	*qMCF-A05-1*	3.22	5.22	−0.01	4015
	E3	A08	52.24	*qMCF-A08-1*	6.49	11.32	0.00	-
	E1	D02	10.92	*qMCF-D02-1*	3.32	10.18	−0.01	4015
	E2	D02	3.37	*qMCF-D02-2*	3.20	10.30	0.01	*G. tomentosum*
	E3	D05	51.40	*qMCF-D05-1*	4.36	7.27	0.00	-
	E3	D07	8.74	*qMCF-D07-1*	4.07	6.74	0.00	-
	E3	D07	21.28	*qMCF-D07-2*	3.48	5.72	0.00	-
FU	E3	A03	27.53	*qFU-A03-1*	6.77	10.86	−0.72	4015
	E1	A05	44.26	*qFU-A05-1*	3.75	7.34	0.92	*G. tomentosum*
	E3	A05	2.79	*qFU-A05-2*	3.54	5.24	−0.26	4105
	E3	A05	78.57	*qFU-A05-3*	8.93	15.03	0.90	*G. tomentosum*
	E1	A06	19.81	*qFU-A06-1*	8.98	19.92	−0.64	4015
	E3	A07	20.64	*qFU-A07-1*	4.86	7.46	−0.34	4015
	E2	A08	88.37	*qFU-A08-1*	3.13	8.04	0.50	*G. tomentosum*
	E2	D08	50.42	*qFU-D08-1*	6.64	18.56	−0.77	4105
	E1	D10	10.63	*qFU-D10-1*	3.49	6.82	−0.36	4105
	E3	D11	16.80	*qFU-D11-1*	6.51	10.41	0.64	*G. tomentosum*
	E1	D12	6.53	*qFU-D12-1*	5.43	11.03	0.63	*G. tomentosum*
	E3	D13	41.37	*qFU-D13-1*	5.90	9.27	−0.76	4015
SFC	E1	A13	59.98	*qSFC-A13-1*	5.46	20.21	0.87	*G. tomentosum*
	E2	D04	50.11	*qSFC-D04-1*	4.13	18.17	1.01	*G. tomentosum*
	E3	D07	53.59	*qSFC-D07-1*	3.84	15.25	−0.48	4015
FE	E2	A03	63.06	*qFE-A03-1*	3.55	8.33	0.49	*G. tomentosum*
	E1	A04	57.88	*qFE-A04-1*	4.82	10.09	0.52	*G. tomentosum*
	E3	A04	10.54	*qFE-A04-2*	3.26	6.32	0.28	*G. tomentosum*
	E2	A05	48.90	*qFE-A05-1*	3.24	7.55	1.51	*G. tomentosum*
	E1	A07	23.81	*qFE-A07-1*	4.56	9.48	−0.32	4015
	E1	A07	71.27	*qFE-A07-2*	6.39	13.86	−1.01	4105
	E2	A07	61.62	*qFE-A07-3*	3.41	7.97	−0.37	4105
	E3	A07	40.54	*qFE-A07-4*	3.75	7.34	0.54	*G. tomentosum*
	E1	A11	93.03	*qFE-A11-1*	3.27	6.60	1.37	*G. tomentosum*
	E2	D04	47.00	*qFE-D04-1*	4.56	10.94	−0.36	4015
	E3	D07	8.93	*qFE-D07-1*	5.60	11.43	−0.38	4015
	E3	D07	21.28	*qFE-D07-2*	7.71	16.50	0.74	*G. tomentosum*
	E2	D10	1.74	*qFE-D10-1*	5.47	13.41	0.42	*G. tomentosum*
SCI	E3	A01	20.34	*qSCI-A01-1*	3.91	6.33	2.82	*G. tomentosum*
	E1	A02	81.75	*qSCI-A02-1*	8.30	20.92	6.84	*G. tomentosum*
	E2	A02	81.75	*qSCI-A02-2*	3.74	14.78	6.66	*G. tomentosum*
	E3	A02	82.67	*qSCI-A02-3*	6.65	11.45	−4.07	4105
	E3	A04	30.63	*qSCI-A04-1*	3.57	5.74	−3.25	4015
	E3	A04	62.70	*qSCI-A04-2*	3.17	5.04	3.18	*G. tomentosum*
	E1	A05	86.26	*qSCI-A05-1*	4.18	9.59	4.71	*G. tomentosum*
	E1	A08	63.85	*qSCI-A08-1*	3.49	7.88	−8.15	4105
	E2	A12	46.99	*qSCI-A12-1*	3.31	12.96	−5.63	4105
	E1	A13	21.67	*qSCI-A13-1*	3.58	8.11	−18.14	4105
	E3	D08	29.17	*qSCI-D08-1*	3.23	5.15	4.86	*G. tomentosum*
	E3	D11	16.80	*qSCI-D11-1*	8.88	16.09	7.29	*G. tomentosum*
	E3	D13	41.37	*qSCI-D13-1*	6.24	10.64	−7.50	4015
TWC	E3	A06	31.94	*qTWC-A06-1*	3.07	12.89	0.20	*G. tomentosum*
	E1	A09	51.56	*qTWC-A09-1*	3.22	8.29	0.19	*G. tomentosum*
	E2	A12	74.65	*qTWC-A12-1*	4.99	12.00	−0.11	4105
	E1	A13	1.76	*qTWC-A13-1*	3.44	8.90	−0.13	4105
	E1	A13	68.49	*qTWC-A13-2*	4.46	11.79	0.13	*G. tomentosum*
	E2	D04	0.35	*qTWC-D04-1*	3.80	8.92	0.09	*G. tomentosum*
	E1	D08	3.82	*qTWC-D08-1*	5.71	15.54	0.21	*G. tomentosum*
	E2	D10	8.18	*qTWC-D10-1*	4.86	11.65	−0.10	4105

Note: the "+" symbol in Add represents that the favorable allele is derived from *G. tomentosum*; the "−" symbol in Add represents that the favorable allele is derived from *G. hirsutum acc.* 4105.

Four QTLs associated with FS (*qFS-A02-1*, *qFS-A02-2*) and SCI (*qSCI-A02-1*, *qSCI-A02-2*), which were detected in more than two environments, were simultaneously detected at the 81.75 Mb locus on chromosome A02. These were considered to be stable QTLs, and these QTLs were positive additive effects, indicating that the favorable alleles were derived from *G. tomentosum*. QTL associated with FL (*qFL-D02-1*), MCF (*qMCF-D02-2*), FE (*qFE-D07-2*), MCF (*qMCF-D07-2*), FS (*qFS-D11-1*), FU (*qFU-D11-1*) and SCI (*qSCI-D11-1*) traits were detected simultaneously on chromosomes D02 (3.37 Mb), D07 (21.28Mb) and D11 (16.8Mb), suggesting that these loci may be tightly linked to fiber quality traits.

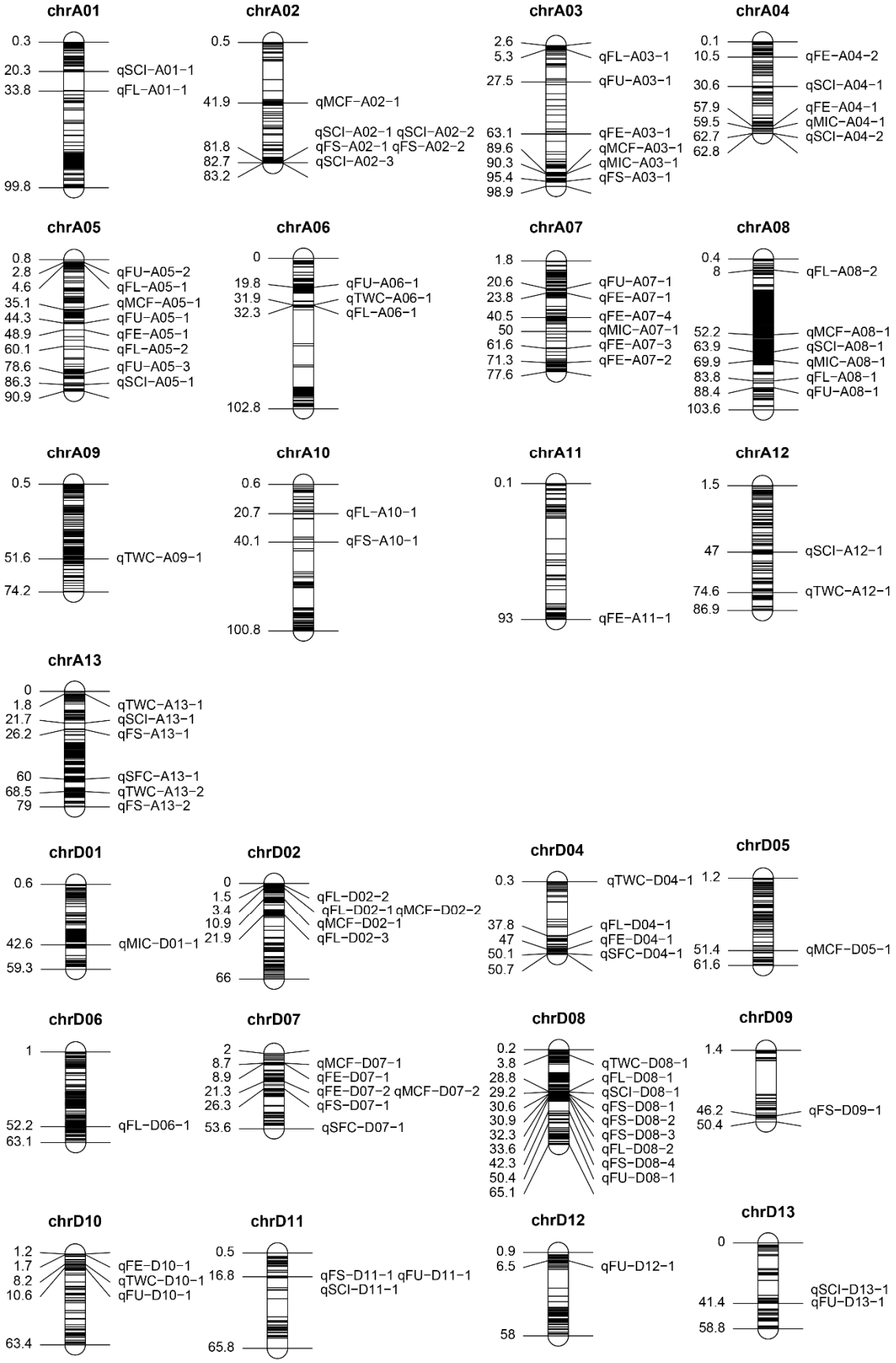

Figure 4. Distribution map of QTLs for fiber quality traits on chromosomes.

Fifteen QTLs for FL were detected on 10 chromosomes (Figure 4; Table 4), and their LOD values ranged from 3.08 to 9.45. The LOD (9.45) value and PVE (16.46%) of *qFL-D02-1* are the highest among all FL-related QTLs, indicating that this locus is closely related to FL.

For FS, thirteen QTLs were detected on eight chromosomes with PVE ranging from 5.64% to 16.71%. Nevertheless, the LOD and PVE values of *qFS-A13-1* were the highest, and the add value was −4.09 (Figure 5), indicating that upland cotton variety 4105 could inhibit FS at this locus. It is worth noting that a stable QTL was simultaneously detected at position 81.75 Mb on chromosome A02, and all showed positive additive effects, indicating that *G. tomentosum* had a positive regulatory effect on FS.

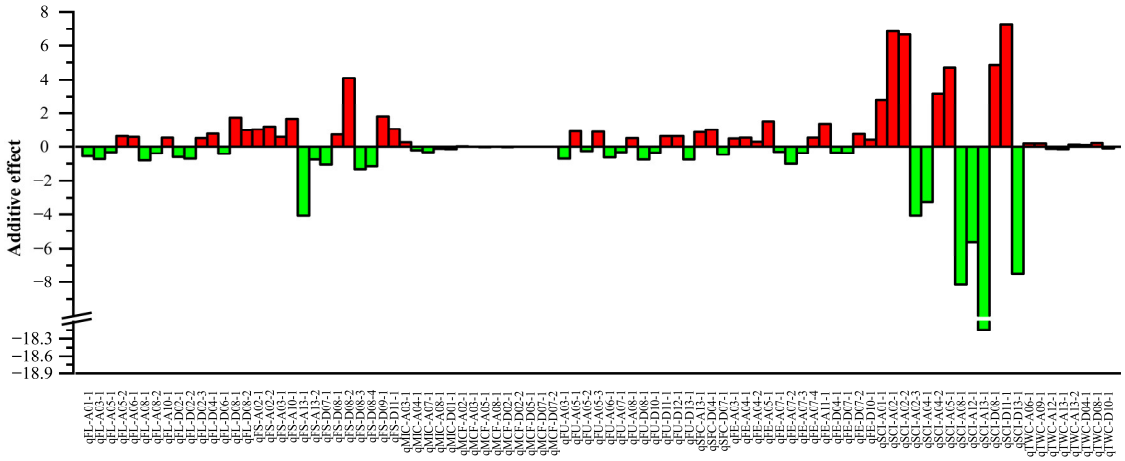

Figure 5. Additive effects of QTLs related to fiber quality traits on chromosomes. Additive effects are displayed on the *y*-axis and QTLs are displayed on the *x*-axis. Red represents a positive additive effect, and the green represents a negative additive effect.

A total of five QTLs were detected by MIC, which was distributed on chromosomes A03, A04, A07, A08 and D01. Four QTLs showed positive additive effects and one QTL showed negative additive effects (Figure 5).

Nine QTLs and twelve QTLs were detected by MCF and FU traits, and the LOD values were 3.2–8.7 and 3.13–8.98, respectively. The PVE was 5.22–16.17% (MCF) and 5.24–19.92% (FU). Four QTLs in MCF had no significant additive effects, and more than half of the QTLs in FU had negative additive effects (Figure 5). However, SFC only localized three QTLs on three chromosomes, which was the least number of QTL traits. LOD and PVE were 3.84–5.46 and 15.25–20.21%, respectively.

Thirteen QTLs were detected in both FE (on eight chromosomes) and SCI (on ten chromosomes). Among them, FE located four QTLs on A07 chromosome (23.81–71.27 Mb), two QTLs (*qFE-A07-2*, *qFE-A07-4*) showed positive additive effects and the other two QTLs (*qFE-A07-1*, *qFE-A07-3*) showed negative additive effects (Figure 5). It is worth mentioning that *qSCI-A02-1* and *qSCI-A02-2* were detected as stable QTLs, and PVE was 14.78%-20.92%, and both showed positive additive effects (Figure 5), which shows that this interval is a tight linkage to SCI traits, and *G. tomentosum* has a positive regulatory role.

In total, eight QTLs were detected for TWC on seven chromosomes (A06, A09, A12, A13, D04, D08 and D10), with PVE ranging from 8.29% to 15.54% for each QTL. Five QTLs are true additive effects and three QTLs are negative additive effects (Figure 5).

3.6. Identification and Gene Expression Analysis of Candidate Genes Relating to Fiber Strength

The FS was mainly affected by cellulose synthesis during the thickening period of the fiber secondary wall, while cell wall biosynthesis and supermolecule structure also affect the cotton fiber strength. Therefore, the genes predominantly expressed in fibers during 20 DPA and 25 DPA were screened, and the genes related to the FS were annotated by gene function as candidate genes.

The QTL (*qFS-A02-1*) locus was detected at the stable point in this study. Confidence intervals were in the upstream and downstream 500 kb intervals at the co-association site A02: 81,750,772 bp. Fourteen genes and annotation information were retrieved from the upland cotton of Huazhong Agricultural University as the reference genome in the Cotton Functional Genomics Database (CottonFGD.org, https://cottonfgd.org/) (accessed on 5 May 2022). Using the retrieved protein sequences of fourteen candidate genes, nine homologous genes were obtained by BLAST analysis of The Arabidopsis Information Resource database (TAIR, https://www.arabidopsis.org/Blast/index.jsp) (accessed on 7 May 2022) (Table S2). *Ghir_A02G012730* encodes Nucleotide-diphospho-sugar transferases protein, and its homologous gene *AT1G64980* (*CDI*) plays a major role in pollen germination and tube growth [32]. Nucleotide-diphospho-sugar transferases were also found in maize to affect the cell wall development of elongated internodes by participating in the transition from primary cell wall to secondary cell wall synthesis [33]. *Ghir_A02G012790* is the gene encoding the cytochrome *P450* protein. Cytochrome *P450* family genes have been declared to be involved in the biosynthesis of lignin precursors or hormone homeostasis, participate in the formation of cell walls and regulate plant growth [34,35]. *Ghir_A02G012830* encodes an evolutionarily conserved protein with putative GTP-binding motifs. Its homologous genes (*AT3G13870*) regulate cell expansion and root hair development [36] while participating in plant-type cell wall biogenesis (Table S2). In Arabidopsis, the *RHD3* gene is required for cell wall biosynthesis and actin organization [37]. These three genes (*Ghir_A02G012730*, *Ghir_A02G012790* and *Ghir_A02G012830*) were identified as candidate genes related to the regulation of cotton fiber strength traits.

To verify the potential role of these genes in fiber strength, the relative expression levels at different fiber developmental stages were analyzed by qRT-PCR technology (Figure 6). The results showed that *Ghir_A02G012730* was preferentially expressed at 0 DPA early in cotton fiber development and strongly expressed again at 25 DPA. Thereafter, its expression level decreased (Figure 6A). The expression pattern of *Ghir_A02G012790* was similar to that of *Ghir_A02G012730* (Figure 6B). The relative expression level of *Ghir_A02G012790* was significantly reduced in 5 DPA and 10 DPA after being preferentially expressed in the early stage, and it was almost not expressed, but it was highly expressed in the late stage of fiber development (20 DPA and 25 DPA). The expression pattern of *Ghir_A02G012830* was expressed throughout the cotton fiber development process and showed an upward trend, and the relative expression level increased rapidly at 20 DPA, and then decreased (Figure 6C).

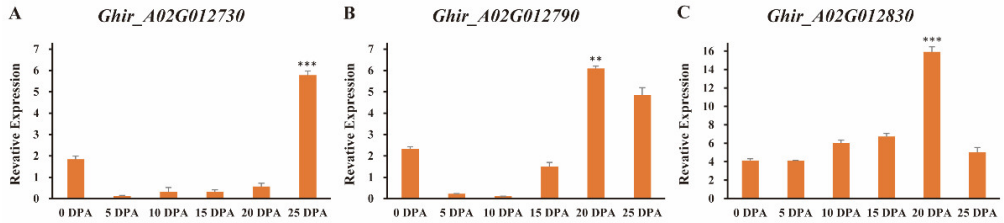

Figure 6. The relative expression levels of the three candidate genes, which is *Ghir_A02G012730* (**A**), *Ghir_A02G012790* (**B**), and *Ghir_A02G012830* (**C**), were obtained on the different stages of cotton fibers in TM-1 by using qRT-PCR. The relative expression levels are shown on the *y*-axis and the different DPA values during fiber development are shown on the *x*-axis. Error bars indicate the standard deviation of three replicates. Significant expression is compared to the previous period. ** significantly correlated at the 0.01 level; *** significantly correlated at the 0.001 level.

So, it is hypothesized that these three genes (*Ghir_A02G012730*, *Ghir_A02G012790* and *Ghir_A02G012830*) play an important role in FS, but the regulating mechanism needs to be further validated.

4. Discussion

4.1. Population Dominance of Wild Species

Upland cotton is the most widely planted cotton species in China, which shows advances in high yield, high boll setting rate and wide adaptability. However, its fiber quality cannot meet the requirements of high-end textiles. In addition, upland cotton has a narrow genetic base, so it is very difficult to improve the fiber quality of upland cotton under the normal breeding procedure. *G. tomentosum* has the characteristics of stress resistance and high fiber strength [13]. Introducing the superior alleles of *G. tomentosum* into upland cotton might enrich the genetic diversity and further improve the potential of fiber quality. At the beginning of the 20th century, the first three-way hybrid was obtained from wild cotton and upland cotton (*G. hirsutum* × *Asiatic cotton* × *G. tomentosum*). Its fiber quality was excellent, but the yield was relatively low. Through continuous backcrossing, hybridization and selective breeding, a cotton variety with excellent fiber quality and high yield (PD875) was cultivated [38]. Subsequently, the favorable alleles derived from *G. tomentosum* regulating non-nectary traits were also successfully introduced into upland cotton [39]. *G. tomentosum* has been crossed with Zhong miansuo 12 to generate F_2 population. A high-density genetic linkage map was constructed, showing that 3093 marks loci were mapped on 26 chromosomes of the cotton genome, with an average distance of 1.48 cM, covering the genetic distance of 4365.3 cM [40]. Zamir described that the population of ILs are an example of the whole exotic genome, where each single line has a chromosome segment from an exotic parent, while the rest of the genome is always obtained from an elite variety [41]. A population of CSSLs with an upland cotton background was constructed by using *G. tomentosum*, and the average length of the introgression homozygous fragment introduced into each chromosome ranged from 0.9 to 9.8 cM, and it was also shown that the introgression fragments had a great influence on FL, FS and MIC traits [16].

In this study, 107 lines of the BC_5S_5 population were constructed using wild tetraploid *G. tomentosum* as donor parents, and stable homozygous lines were obtained through backcrossing, which provided genetic resources for further identification. In terms of fiber quality traits, the population showed a large range of variation, and the introduction fragments of *G. tomentosum* effectively affected the fiber quality traits such as FS, SFC and so on. The QTL mapping of fiber quality was carried out for the BC_5S_5 population, which lay the foundation for the follow-up study of fine mapping and function of QTL.

4.2. Sources and Effects of Favorable Alleles

In this study, *G. hirsutum acc*. 4105 was used as the recurrent parent and *G. tomentosum* as the donor parent. In all QTLs detected, forty-six favorable alleles of QTLs were from *G. tomentosum* and forty-one favorable alleles of QTLs were from *G. hirsutum acc*. 4105. The favorable alleles were mainly derived from *G. hirsutum acc*. 4105, including FL, MIC and FU, which contained seven, one and five QTLs, respectively. In quality traits such as FS, MCF, SFC, FE, SCI and TWC, most of the favorable alleles come from *G. tomentosum*. The results show that the introduction of *G. tomentosum* fragments can help to improve the fiber quality traits such as FS, FE and SFC, which is consistent with the fine and strong fiber quality of *G. tomentosum* and is of great significant research value for the improvement of upland cotton varieties. It is worth noting that among the stable QTLs (*qFS-A02-1* and *qSCI-A02-1*) detected, the favorable alleles were all derived from *G. tomentosum* (Table 4; Figure 7).

In summary, QTL-favorable alleles derived from *G. tomentosum* had a great impact on FS, SFC, SCI and other traits, which play a certain role in improving the fiber quality traits of upland cotton. These results indicated that the favorable alleles of the QTL come from high-value parents and low-value phenotypic parents. This is consistent with the previous research [42,43].

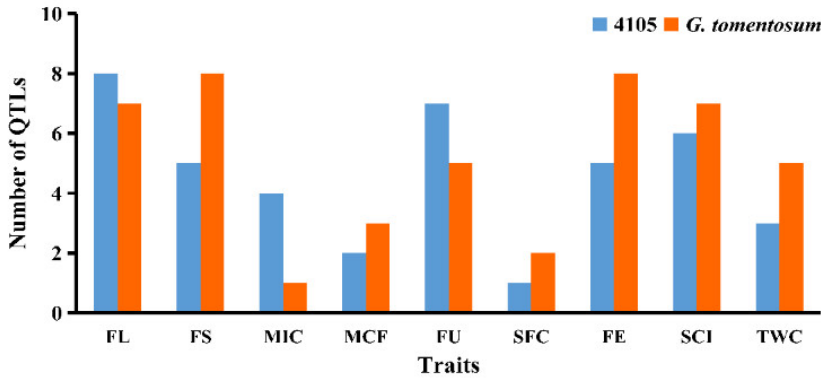

Figure 7. The distribution of the source for the favorable alleles regulating fiber quality traits.

4.3. Distribution of QTL Clusters

Most QTLs related to cotton yield and fiber quality traits were clustered and co-linked [44]. A region containing three or more QTLs (which can be used to control different traits) within 20 cM of the chromosome is called a QTL cluster. It is estimated that the average 1 cM of cotton genome is equivalent to the physical region of 0.5 Mb [45]. The distribution of six QTL clusters was also found in this study (Table 5, Figure 4), namely A02-cluster, A03-cluster, A04-cluster, D02-cluster, D07-cluster and D08-cluster. These QTL clusters included quantitative traits of FS, SCI, MCF, MIC, FE and FL, among which the number of QTLs in D08-cluster was the largest (has six QTLs).

Table 5. QTL cluster distribution of fiber quality traits on chromosome.

QTL Clusters	Chromosome Position Range (Mb)	QTL Number	QTL
A02-cluster	81.75–82.67	5	*qFS-A02-1*, *qFS-A02-2* *qSCI-A02-1*, *qSCI-A02-2* *qSCI-A02-3*
A03-cluster	89.63–95.37	3	*qMCF-A03-1*, *qMIC-A03-1* *qFS-A03-1*
A04-cluster	57.88–62.70	3	*qFE-A04-1*, *qMIC-A04-1* *qSCI-A04-2*
D02-cluster	1.50–10.92	4	*qFL-D02-1*, *qFL-D02-2* *qMCF-D02-1*, *qMCF-D02-2*
D07-cluster	21.28–26.27	3	*qMCF-D07-2*, *qFE-D07-2* *qFS-D07-1*
D08-cluster	28.79–33.59	6	*qFL-D08-1*, *qFL-D08-2* *qSCI-D08-1*, *qFS-D08-1* *qFS-D08-2*, *qFS-D08-3*

In previous studies, ten QTL clusters were found on chromosomes A01, A03, A07, A09, A11, D05, D06, D07 and D08 [46]. In this study, QTL clusters were also found on chromosomes of A03, D07 and D08, and their physical locations were similar to those of their predecessors. Using the BC_5S_5 population for QTL mapping, five QTL clusters were found on chromosomes A01, A09, A13, D02 and D10 [15]. The same QTL clusters were not found on other chromosomes, which may be due to the influence of the number of loci and the environment. These QTL clusters may be associated with multiple fiber quality traits of cotton at the same time, which may be controlled by one polymorphism gene or multiple closely linked genes [4].

4.4. Analysis of Co-Location QTL and Stable QTL

The genetic basis of cotton fiber quality traits is complex and easily influenced by genes and environment. QTLs with stable and high effect value can be used in cotton molecular-assisted breeding to promote cotton genetic improvement [47]. Stable QTLs can be detected in two or more environments [27]. In this study, 91 QTLs related to fiber quality traits were detected and compared with the CottonQTLdb.org (http://www2.cottonqtldb.org) database (accessed on 20 June 2022) [47], and the genetic distance on the chromosome was determined by using markers on both sides of the QTL locus. QTL mapping of fiber quality traits was achieved using RIL population. A total of sixty-two QTLs were detected to be associated with fiber quality traits, among which *qFL05.1* (marker: NAU2894) has a similar physical location to *qFL-A05-1* (4,582,887 bp), and its physical location is ChrA05: 4,627,546–4,627,708 bp [48]. A genome-wide association analysis (GWAS) was performed for sixteen traits (fiber quality and yield) in a natural population of 503 materials. A total of 160 QTLs were detected. *qGhFE-c22-1* (marker: i12840Gh) and *qGhFS-c2-1* (marker: i03136Gh) were close to the physical locations of *qFE-D04-1* (47,001,754 bp) and *qFS-A02-1* (81,750,772 bp), and their physical locations are ChrD04: 46,646,103–48,654,115 bp and ChrA02: 81,837,208–82,737,585 bp, respectively [49]. Diouf used the $F_{2:3}$ population of 277 families for QTL mapping and detected a total of 110 QTLs associated with fiber quality and yield traits. *qFS-A02-1* (81,750,772 bp) stably detected in this study may be related to *qFS-A02-15* (marker: mk1761_A02—mk1778_A02) and *qFS-A02-cb* (marker: mk1761_A02-mk1778_A02), whose physical positions were ChrA02: 80,488,799–81,766,125 bp; *qSCI-A02-1* (81,750,772 bp) was close to *qSCI-A02-15* (marker: mk1761_A02-mk1778_A02), and *qSCI-A02-cb* (marker: mk1761_A02-mk1778_A02) [50]. Fiber quality traits were mapped by QTL using two RIL populations and backcross populations. The physical position of *qFS-Chr02-1* (marker: ICR11064—MGHES0024) detected in this study was similar to that of *qFS-A02-1* (81,750,772 bp) stably detected in this study, whose physical position was ChrA02: 82,552,263–82,552,476 bp [51].

In summary, the results of QTL detected in this study are feasible. *qFS-A02-1* and *qSCI-A02-1* were located on the same interval of the chromosome, were stably detected in both environments and acquired the favorable alleles from donor parents. The stable QTLs detected in this study can be further applied to cotton molecular design breeding and promote precise genetic improvements in upland cotton.

4.5. Candidate Gene Expression Analysis

In this study, three genes related to cotton fiber development were obtained, and the expressions of these three genes were up-regulated on the 20th to 25th day after cotton fiber development. These three genes were related to cell elongation or lignin synthesis. Cotton fiber is related to the elongation of surface cells and the thickening of secondary walls. The development pattern of cotton fiber verified the effect of candidate genes on cotton quality. In this study, TM-1 was used to verify the expression levels of three candidate genes. The effects of different genotypes of these three candidate genes on cotton fiber development need to be further verified.

5. Conclusions

In this study, ninety-one QTL loci related to fiber quality traits were detected in a BC_5S_5 upland population of 107 lines, which were distributed on 25 chromatins. Additionally, the PEV rangedfrom 4.53% to 20.92%. More than half of the favorable alleles of QTL derived from *G. tomentosum*. Among them, the favorable alleles of QTLs (*qFS-A02-1* and *qSCI-A02-1*) in stable detection were all from *G. tomentosum*, and the PVE was 9.8–16.71% and 14.78–20.92%, respectively, indicating that the introduction fragment of *G. tomentosum* greatly improved the fiber quality traits of upland cotton. Fourteen genes were retrieved in the candidate interval, and it was found that *Ghir_A02G012730*, *Ghir_A02G012790* and *Ghir_A02G012830* may be involved in cellulose and cell wall biosynthesis and had a relatively high expression during fiber development (20 DPA and 25 DPA). This suggests

that these three genes may be involved in the regulation of fiber strength traits, but their functions need further verification to determine the regulation mechanism.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture13030579/s1. Supplementary Table S1. Primers of qRT-PCR; Supplementary Table S2. Related genes and annotation information in the interval; Supplementary Figure S1. BC_5S_5 introduction line population construction; Supplementary Figure S2. Phenotypic distribution of nine fiber quality traits in the ILs across three environments; Supplementary Figure S3. Number of SNP markers in chromosomes; Supplementary Figure S4. SNP genetic linkage map of BC_5S_5 population; Supplementary Figure S5. Genotypes of 107 ILs (gray and red indicate the 4015 and *G. tomentosum*, respectively); Supplementary Figure S6. Distribution of QTL of fiber quality trait QTL on chromosomes.

Author Contributions: X.N., Z.L. and J.X. designed the experiments and provided the cotton germplasm resources. X.C., C.G., Z.P., Y.W., L.C., G.S. and C.Y. performed the experiments. X.C. and C.S. performed the data analysis. X.C. finished the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fund for the Innovation Leadership Program in Sciences and Technologies for Scientists of Xinjiang production and Construction Corps, China (S2019CB1877); Innovation Leadership Program in Sciences and Technologies for Young and Middle-aged Scientists of Xinjiang production and Construction Corps, China (2021CB028); the Key Programs for Science and Technology Development of Shihezi city, Xinjiang production and Construction Corps, China (2022NY01); the Key Programs for Science and Technology Development of Shihezi city, Xinjiang production and Construction Corps, China (2021NY01); and Innovation Leadership Program in Sciences and Technologies for Scientists of Fifth Division, Xinjiang production and Construction Corps, China (2021NY02).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The genome sequences of ILs developed from SLAF-seq are available at NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/bioproject/, accessed on 6 December 2022) with BioProject accession number PRJNA421265 and PRJNA316549.

Conflicts of Interest: All authors declare that they have no conflict of interest.

References

1. Wang, M.; Tu, L.; Yuan, D.; Zhu, D.; Shen, C.; Li, J.; Liu, F.; Pei, L.; Wang, P.; Zhao, G.; et al. Reference genome sequences of two cultivated allotetraploid cottons, *Gossypium hirsutum* and *Gossypium barbadense*. *Nat. Genet.* **2019**, *51*, 224–229. [CrossRef] [PubMed]
2. Fang, D.D.; Jenkins, J.N.; Deng, D.D.; McCarty, J.C.; Li, P.; Wu, J. Quantitative trait loci analysis of fiber quality traits using a random-mated recombinant inbred population in Upland cotton (*Gossypium hirsutum* L.). *BMC Genom.* **2014**, *15*, 397. [CrossRef] [PubMed]
3. Gore, M.A.; Fang, D.D.; Poland, J.A.; Zhang, J.; Percy, R.G.; Cantrell, R.G.; Thyssen, G.; Lipka, A.E. Linkage Map Construction and Quantitative Trait Locus Analysis of Agronomic and Fiber Quality Traits in Cotton. *Plant Genome* **2015**, *7*, plantgenome2013.07.0023. [CrossRef]
4. Said, J.I.; Lin, Z.; Zhang, X.; Song, M.; Zhang, J. A comprehensive meta QTL analysis for fiber quality, yield, yield related and morphological traits, drought tolerance, and disease resistance in tetraploid cotton. *BMC Genom.* **2013**, *14*, 776. [CrossRef]
5. Ye, S.H.; Wu, D.X.; Lu, X.J.; Tian, C.J.; Pan, Z.J.; Yang, H.; Wu, C. Research progress on QTL positioning of cotton fiber quality. *Cotton Sci.* **2017**, *39*, 2–6. [CrossRef]
6. Lander, E.S.; Botstein, D. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **1989**, *121*, 185–199. [CrossRef]
7. Wang, K.; Wang, Z.; Li, F.; Ye, W.; Wang, J.; Song, G.; Yue, Z.; Cong, L.; Shang, H.; Zhu, S.; et al. The draft genome of a diploid cotton *Gossypium raimondii*. *Nat. Genet.* **2012**, *44*, 1098–1103. [CrossRef]
8. Li, F.; Fan, G.; Wang, K.; Sun, F.; Yuan, Y.; Song, G.; Li, Q.; Ma, Z.; Lu, C.; Zou, C.; et al. Genome sequence of the cultivated cotton *Gossypium arboreum*. *Nat. Genet.* **2014**, *46*, 567–572. [CrossRef]
9. Zhang, T.; Hu, Y.; Jiang, W.; Fang, L.; Guan, X.; Chen, J.; Zhang, J.; Saski, C.A.; Scheffler, B.E.; Stelly, D.M.; et al. Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. acc. TM-1) provides a resource for fiber improvement. *Nat. Biotechnol.* **2015**, *33*, 531–537. [CrossRef]

10. Liu, X.; Zhao, B.; Zheng, H.J.; Hu, Y.; Lu, G.; Yang, C.Q.; Chen, J.D.; Chen, J.J.; Chen, D.Y.; Zhang, L.; et al. *Gossypium barbadense* genome sequence provides insight into the evolution of extra-long staple fiber and specialized metabolites. *Sci. Rep.* **2015**, *5*, 14139. [CrossRef]
11. Wang, B.; Nie, Y.; Lin, Z.; Zhang, X.; Liu, J.; Bai, J. Molecular diversity, genomic constitution, and QTL mapping of fiber quality by mapped SSRs in introgression lines derived from *Gossypium hirsutum* × *G. darwinii* Watt. *Theor. Appl. Genet.* **2012**, *125*, 1263–1274. [CrossRef]
12. Shen, C.; Ding-Guo, L.I.; Nie, Y.C.; Lin, Z.X. QTL Mapping for Yield and Fiber Quality Traits Using *Gossypium mustelinum* Chromosome Segment Introgression Lines. *Acta Agron. Sin.* **2017**, *43*, 1733. [CrossRef]
13. Hou, M.; Cai, C.; Zhang, S.; Guo, W.; Zhang, T.; Zhou, B. Construction of microsatellite-based linkage map and mapping of nectarilessness and hairiness genes in *Gossypium tomentosum*. *J. Genet.* **2013**, *92*, 445–459. [CrossRef]
14. Zhang, Z.; Rong, J.; Waghmare, V.N.; Chee, P.W.; May, O.L.; Wright, R.J.; Gannaway, J.R.; Paterson, A.H. QTL alleles for improved fiber quality from a wild *Hawaiian* cotton, *Gossypium tomentosum*. *Theor. Appl. Genet.* **2011**, *123*, 1075. [CrossRef]
15. Keerio, A.A.; Shen, C.; Nie, Y.; Ahmed, M.M.; Zhang, X.; Lin, Z. QTL Mapping for Fiber Quality and Yield Traits Based on Introgression Lines Derived from *Gossypium hirsutum* × *G. tomentosum*. *Int. J. Mol. Sci.* **2018**, *19*, 10243. [CrossRef]
16. Hao, Y.S. QTL Mapping for Yield and Fiber Quality Traits from the Chromosome Segment Substitution Lines of *Gossypium tomentosum*. Master's Thesis, Southwest University, Chongqing, China, 2020.
17. Zhao, M.; Zhou, H.; Luo, Y.; Wang, J.; Hu, J.; Liu, X.; Li, S.; Zhang, K.; Zhen, H.; Hickford, J.G.H. Variation in a Newly Identified Caprine KRTAP Gene Is Associated with Raw Cashmere Fiber Weight in Longdong Cashmere Goats. *Genes* **2021**, *12*, 625. [CrossRef]
18. Vélez, J.M.; Morris, R.M.; Vilgalys, R.; Labbé, J.; Schadt, C.W. Phylogenetic diversity of 200+ isolates of the ectomycorrhizal fungus *Cenococcum geophilum* associated with *Populus trichocarpa* soils in the Pacific Northwest, USA and comparison to globally distributed representatives. *PLoS ONE* **2021**, *16*, e0231367. [CrossRef]
19. Zhao, X.; Yu, K.; Pang, C.; Wu, X.; Shi, R.; Sun, C.; Zhang, W.; Chen, F.; Zhang, J.; Wang, X. QTL Analysis of Five Silique-Related Traits in *Brassica napus* L. across Multiple Environments. *Front. Plant Sci.* **2021**, *12*, 766271. [CrossRef]
20. Shen, C.; Jin, X.; Zhu, D.; Lin, Z. Uncovering SNP and indel variations of tetraploid cottons by SLAF-seq. *BMC Genom.* **2017**, *18*, 247. [CrossRef]
21. Sun, X.; Liu, D.; Zhang, X.; Li, W.; Liu, H.; Hong, W.; Jiang, C.; Guan, N.; Ma, C.; Zeng, H.; et al. SLAF-seq: An efficient method of large-scale de novo SNP discovery and genotyping using high-throughput sequencing. *PLoS ONE* **2013**, *8*, e58700. [CrossRef]
22. Li, H.; Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **2009**, *25*, 1754–1760. [CrossRef] [PubMed]
23. Lu, Q.; Xiao, X.; Gong, J.; Li, P.; Zhao, Y.; Feng, J.; Peng, R.; Shi, Y.; Yuan, Y. Identification of Candidate Cotton Genes Associated with Fiber Length Through Quantitative Trait Loci Mapping and RNA-Sequencing Using a Chromosome Segment Substitution Line. *Front. Plant Sci.* **2021**, *12*, 796722. [CrossRef] [PubMed]
24. Wang, J.; Wan, X.; Li, H.; Pfeiffer, W.H.; Crouch, J.; Wan, J. Application of identified QTL-marker associations in rice quality improvement through a design-breeding approach. *Theor. Appl. Genet.* **2007**, *115*, 87–100. [CrossRef] [PubMed]
25. Ouellette, L.A.; Reid, R.W.; Blanchard, S.G.; Brouwer, C.R. LinkageMapView-rendering high-resolution linkage and QTL maps. *Bioinformatics* **2018**, *34*, 306–307. [CrossRef]
26. Mccouch, S.; Cho, Y.; Yano, M.; Paul, E.; Blinstrub, M.; Morishima, H.; Mccouch, S.; Cho, Y.; Paul, E.; Morishima, H. Report on QTL nomenclature. In *Rice Genetics Newsletter*; Science Open: Berlin, Germany, 1997; Volume 14, pp. 11–13.
27. Wang, B.; Liu, L.; Zhang, D.; Zhuang, Z.; Guo, H.; Qiao, X.; Wei, L.; Rong, J.; May, O.L.; Paterson, A.H.; et al. A Genetic Map Between *Gossypium hirsutum* and the Brazilian Endemic *G. mustelinum* and Its Application to QTL Mapping. *G3 Genes Genomes Genet.* **2016**, *6*, 1673–1685. [CrossRef]
28. Guo, A.H.; Su, Y.; Huang, Y.; Wang, Y.M.; Nie, H.S.; Zhao, N.; Hua, J.P. QTL controlling fiber quality traits under salt stress in upland cotton (*Gossypium hirsutum* L.). *Theor. Appl. Genet.* **2021**, *134*, 661–685. [CrossRef]
29. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
30. Reyes, B. Genomic and epigenomic bases of transgressive segregation—New breeding paradigm for novel plant phenotypes. *Plant Sci. Int. J. Exp. Plant Biol.* **2019**, *288*, 110213. [CrossRef]
31. Ooijen, J. *JoinMap 4.0: Software for the Calculation of Genetic Linkage Maps in Experimental Population*; Kyazma BV: Wageningen, The Netherlands, 2006.
32. Li, H.M.; Chen, H.; Yang, Z.N.; Gong, J.M. *Cdi* gene is required for pollen germination and tube growth in *Arabidopsis*. *FEBS Lett.* **2012**, *586*, 1027–1031. [CrossRef]
33. Peng, C.; Wang, X.; Feng, T.; He, R.; Zhang, M.; Li, Z.; Zhou, Y.; Duan, L. System Analysis of MIRNAs in Maize Internode Elongation. *Biomolecules* **2019**, *9*, 417. [CrossRef]
34. Nelson, D.; Werck-Reichhart, D. A *P450*-centric view of plant evolution. *Plant J. Cell Mol. Biol.* **2011**, *66*, 194–211. [CrossRef]
35. Tamiru, M.; Undan, J.R.; Takagi, H.; Abe, A.; Yoshida, K.; Undan, J.Q.; Natsume, S.; Uemura, A.; Saitoh, H.; Matsumura, H.; et al. A cytochrome *P450*, *OsDSS1*, is involved in growth and drought stress responses in rice (*Oryza sativa* L.). *Plant Mol. Biol.* **2015**, *88*, 85–99. [CrossRef]

36. Wang, H.; Lee, M.M.; Schiefelbein, J.W. Regulation of the cell expansion gene *RHD3* during *Arabidopsis* development. *Plant Physiol.* **2002**, *129*, 638–649. [CrossRef]
37. Hu, Y.; Zhong, R.; Morrisoniii, W.H.; Ye, Z.H. The *Arabidopsis RHD3* gene is required for cell wall biosynthesis and actin organization. *Planta* **2003**, *217*, 912–921. [CrossRef]
38. Beasley, J. The Origin of American Tetraploid *Gossypium* Species. *Am. Nat.* **1940**, *74*, 285–286. [CrossRef]
39. Meyer, V.G.; Meredith, W.R. New germplasm from crossing Upland cotton (*Gossypium hirsutum*) with *G. tomentosum*. *J. Hered.* **1978**, *69*, 183–187. [CrossRef]
40. Khan, A.I.; Awan, F.S.; Sadia, B.; Rana, R.M.; Khan, I.A. Genetic diversity studies among coloured cotton genotypes by using RAPD markers. *Pak. J. Bot.* **2010**, *42*, 71–77. [CrossRef]
41. Zamir, D. Improving plant breeding with exotic genetic libraries. *Nat. Rev. Genet.* **2001**, *2*, 983–989. [CrossRef]
42. Lan, M.J. Characterization of Donor Chromosome Fragments from *Gossypium barbadense* L. in CCRI45 Background of *Gossypium hirsutum* L. and Identification of QTL Related to Fiber Yield and Quality Traits. Master's Thesis, Chinese Academy of Agricultural Sciences, Beijing, China, 2011.
43. Shen, X.; Guo, W.; Lu, Q.; Zhu, X.; Yuan, Y.; Zhang, T. Genetic mapping of quantitative trait loci for fiber quality and yield trait by RIL approach in Upland cotton. *Euphytica* **2007**, *155*, 371–380. [CrossRef]
44. Zhang, J.F.; Dan, Y.Z.; LIANG, Y.; Gu, Y.J.; Zhang, B.C.; Li, J.W.; Gong, J.W.; Liu, A.Y.; Shang, H.G.; Wang, T. Evaluation of Yield and Fiber Quality Traits of Chromosome Segment Substitution Lines Population (BC_5F_3 and BC_5F_{34}) in Cotton. *J. Plant Genet. Resour.* **2012**, *13*, 773–781. [CrossRef]
45. Li, X.; Jin, X.; Wang, H.; Zhang, X.; Lin, Z. Structure, evolution, and comparative genomics of tetraploid cotton based on a high-density genetic linkage map. *DNA Res. Int. J. Rapid Publ. Rep. Genes Genomes* **2016**, *23*, 283–293. [CrossRef] [PubMed]
46. Yang, X.; Zhou, X.; Wang, X.; Li, Z.; Zhang, Y. Mapping QTL for cotton fiber quality traits using simple sequence repeat markers, conserved intron-scanning primers, and transcript-derived fragments. *Euphytica* **2015**, *201*, 215–230. [CrossRef]
47. Liu, R.; Gong, J.; Xiao, X.; Zhang, Z.; Li, J.; Liu, A.; Lu, Q.; Shang, H.; Shi, Y.; Ge, Q.; et al. GWAS Analysis and QTL Identification of Fiber Quality Traits and Yield Components in Upland Cotton Using Enriched High-Density SNP Markers. *Front. Plant Sci.* **2018**, *9*, 1067. [CrossRef] [PubMed]
48. Tang, S.; Teng, Z.; Zhai, T.; Fang, X.; Liu, F.; Liu, D.; Zhang, J.; Liu, D.; Wang, S.; Zhang, K. Construction of genetic map and QTL analysis of fiber quality traits for Upland cotton (*Gossypium hirsutum* L.). *Euphytica* **2015**, *201*, 195–213. [CrossRef]
49. Huang, C.; Nie, X.; Shen, C.; You, C.; Li, W.; Zhao, W.; Zhang, X.; Lin, Z. Population structure and genetic basis of the agronomic traits of upland cotton in China revealed by a genome-wide association study using high-density SNPs. *Plant Biotechnol. J.* **2017**, *15*, 1374–1386. [CrossRef]
50. Diouf, L.; Magwanga, R.O.; Gong, W.; He, S.; Pan, Z.; Jia, Y.H.; Kirungu, J.N.; Du, X. QTL Mapping of Fiber Quality and Yield-Related Traits in an Intra-Specific Upland Cotton Using Genotype by Sequencing (GBS). *Int. J. Mol. Sci.* **2018**, *19*, 20441. [CrossRef]
51. Shang, L.; Wang, Y.; Wang, X.; Liu, F.; Abduweli, A.; Cai, S.; Li, Y.; Ma, L.; Wang, K.; Hua, J. Genetic Analysis and QTL Detection on Fiber Traits Using Two Recombinant Inbred Lines and Their Backcross Populations in Upland Cotton. *G3 Genes Genomes Genet.* **2016**, *6*, 2717–2724. [CrossRef]