QTL controlling fiber quality traits under salt stress in upland cotton (*Gossypium hirsutum* L.)

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Abstract

**Key message** QTL for fiber quality traits under salt stress discerned candidate genes controlling fatty acid metabolism.

**Abstract** Salinity stress seriously affects plant growth and limits agricultural productivity of crop plants. To dissect the genetic basis of response to salinity stress, a recombinant inbred line population was developed to compare fiber quality in upland cotton (*Gossypium hirsutum* L.) under salt stress and normal conditions. Based on three datasets of (1) salt stress, (2) normal growth, and (3) the difference value between salt stress and normal conditions, 51, 70, and 53 QTL were mapped, respectively. Three QTL for fiber length (FL) (*qFL-Chr1-1*, *qFL-Chr5-5*, and *qFL-Chr24-4*) were detected under both salt and normal conditions and explained 4.26%, 9.38%, and 3.87% of average phenotypic variation, respectively. Seven genes within intervals of two stable QTL (*qFL-Chr1-1* and *qFL-Chr5-5*) were highly expressed in lines with extreme long fiber. A total of 35 QTL clusters comprised of 107 QTL were located on 18 chromosomes and exhibited pleiotropic effects. Thereinto, two clusters were responsible for improving five fiber quality traits, and 6 influenced FL and fiber strength (FS). The QTL with positive effect for fiber length exhibited active effects on fatty acid synthesis and elongation, but the ones with negative effect played passive roles on fatty acid degradation under salt stress.

Introduction

Soil salinity, as one of the major abiotic stresses, reduces global agricultural productivity due to the harmful effects on plant growth. Two main approaches can be used to produce salt-tolerant crops: (i) exploitation of natural genetic variations by direct selection or mapping quantitative trait loci (QTL) for subsequent marker-assisted selection (MAS); (ii) generation of transgenic plants to affect the degree of salt stress tolerance (Yamaguchi and Blumwald 2005). Salt stress is known to repress plant growth due to osmotic stress, which is then followed by ion toxicity. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest, and even death (Flowers 2004; Zhu 2001; Wang and Huang 2019; Richter et al. 2019). To overcome salt stress, complicated adjustment to metabolic networks with multipronged responses involves fast-acting, immediate physiological responses, and long-term reactions has been highlighted in plants under high salinity conditions (Guo et al. 2015; Zhang et al. 2011; Liu et al. 2019). Complex multi-component signaling pathways in plants can be triggered face salinity stress, such as plant hormones, plant transcription factor families (Su et al. 2017a), lipids (Gao et al. 2019), aquaporins (Wang et al. 2019; Pawlowicz and Masajada 2019), CIPK(Ma et al. 2019a, b), Snf (sucrose non-fermenting)-1-related protein kinases (SnRK2) (Su et al. 2017b; Shinozawa et al. 2019), and mitogen-activated protein kinase (MAPK), ROS signaling (Ismail and Horie 2017; Wang and Huang 2019) and stomatal signaling (Goldlack et al. 2014).

Previous studies investigating salt tolerance was performed in QTL mapping at the germination stage or during...
vegetative growth, such as in cotton (Diouf et al. 2017; Sun et al. 2018, 2019), rice (Kumar et al. 2015), barley (Mano and Takeda 1997), tomato (Foolad and Chen 1999), and soybean (Lee et al. 2004). A major QTL for salt tolerance in soybean was discovered in 106 F2-derived lines from the cross of ‘S-100’ (salt tolerant line) × ‘Tokyo’ (salt sensitive line) with 41% of total genetic variation for salt tolerance in the field (Lee et al. 2004). The gene (HKT1;4-A2) was identified responsible to salt tolerance based on the QTL Nax1, which was mapped as a salt stress related QTL and linked to the microsatellite marker gwm312 on chromosome 2AL in durum wheat (Triticum monococcum) in durum wheat (Triticum monococcum) (Huang et al. 2006; Tounsi et al. 2016). Therefore, more researches involving QTL exploration salt tolerance are imperative for further development in crop.

The damaging effects of salt accumulation in agricultural soils affect crop productivity due to soil salinity. Upland cotton, as an important crop for renewable natural fiber source of textiles, is a pioneer crop in saline areas, because it is tolerant with salinity threshold of 7.7 dS/m (77 mM NaCl), higher than soil saline with 4 dS/m (40 mM NaCl) (Chinnusamy et al. 2006; Shi et al. 2015). The release of genome data of upland cotton has greatly facilitated cotton research (Zhang et al. 2015; Hu et al. 2019a, b; Chen et al. 2020). Cultivated upland cotton adapted to various environments and experienced periodic salinity extremes (Wendel et al. 2010), originating from D sub-genome of response to abiotic stresses (Zhang et al. 2010). Eight SSR (simple sequence repeats) sites significantly associated with salt tolerance were found at the seedling stage through an association analysis in 134 cotton cultivars (Zhao et al. 2016). Comparative transcriptome analysis revealed that gas signaling process and ROS responding process enhanced abiotic stress in domesticated cotton and prolonged the duration of fiber elongation (Chaudhary et al. 2008, 2009; Park et al. 2012). One hundred and twenty-eight of the early differentially expressed proteins (DEPs) were identified from salt-treated cotton roots, 76 of which displayed increased abundance and 52 decreased under salt stress conditions in upland cotton (Li et al. 2015).

Obviously more exploration is needed in cotton, since no QTL under salt stress condition is included in 551 cotton QTL identified in Cottongen database resource (https://www.cottongen.org) (Ijaz et al. 2019). Some QTL clusters and hotspots containing 661 QTL were collected for traits response to salt stress in cotton, of which, 80 QTL were detected for salt tolerance just in greenhouse condition but no QTL in field condition (Abdelraheem et al. 2017). A total of 11 consistent QTL were detected for seven traits in seedling stage in an F2.3 population at 150 mM NaCl in a hydroponic environment (Oluoch et al. 2016). Recently, nine candidate intron length polymorphisms (ILPs) markers were verified using association mapping in a set of natural upland cotton accessions for salt stress in greenhouse condition (Cai et al. 2017). And the C4 gene encoding WRKY DNA-binding protein and the C9 gene encoding mitogen-activated protein kinase can significantly enhance cotton susceptibility to salt stress. The assessment of stress tolerance in the greenhouse often has little correlation with tolerance in the field (Yamaguchi and Blumwald 2005).

Cotton fiber is one of the most prevalent natural materials used in textile production. Fiber development consists of four stages (initiation, elongation, secondary cell wall biosynthesis, and maturation), which are defined and based on the number of days post-anthesis (DPA). For fiber development, the most active stage is the rapid elongation stage following the initiation stage and lasts up to 20 DPA (Kim et al. 2001). Studies have shown that the biosynthesis of very-long-chain fatty acids (VLCFAs, fatty acids > C18) plays important roles in fiber development as well as the precursors of sphingolipids, seed triacylglycerols, suberin, and cuticular waxes (Qin et al. 2007; Qin and Zhu 2011; Hu et al. 2019a, b). VLCFAs may activate ethylene synthesis in cotton fiber elongation (Shi et al. 2006). KCS (3-ketoacyl-CoA synthase) is the first limiting enzyme in the biosynthesis steps of VLCFA, which determines the substrate and tissue specificities of the reaction in plants (Qin et al. 2007). It is reported that 21 KCS genes were identified in the Arabidopsis genome with distinct tissue-specific, temporal-specific or spatial-specific expression patterns, reflecting their multiple roles in plant growth and development (Qin and Zhu 2011).

There is a lack of experimentation under natural salt stress conditions in the current literature. In the study, we evaluated field performance of five fiber quality traits under two conditions using a recombinant inbred lines (RIL) population derived from a cross of ‘Xinza 1’, an F1 hybrid upland cotton cultivar upland cotton. We performed QTL mapping of fiber quality traits under salt stress and normal growth conditions in three years in order to explore genetic basis of fiber development under salt stress in upland cotton. The findings of this research identify candidate genes associated with fiber elongation underlying the QTL and provide valuable insights for the improvement of cotton fiber quality.

**Materials and methods**

**Plant materials**

The RIL population was derived from a F1 hybrid ‘Xinza 1’ (GX1135 × GX100-2) by single seed descent method in upland cotton (Shang et al. 2015, 2016a), including 177 lines of F15–F17 generations. The control set was performed in two field trials, including GX1135, ‘Xinza 1′ F1, GX100-2, and a commercial hybrid ‘Ruiza 816’ used as a competitive check (Ma et al. 2017, 2019a, b).
Field arrangement

Two field trials under salt stress and normal conditions were conducted in 2016, 2017, and 2018 at Quzhou Experimental Station of China Agricultural University, Handan City, Hebei Province (36°78′N, 114°92′E). Quzhou County is well known for the achievements in saline-alkali soil improvement since 1970′s.

Two independent field trials were arranged in neighboring fields following a randomized complete block design with two replications each in 2016, 2017 and 2018. A total of 362 plots with two rows (22 individual plants per row) were conducted, respectively. Two repeats of 177 RI Lines (F_{15}–F_{17}) were planted together with two control sets (GX1135, F_{1} ‘Xinza 1’, GX100-2, ‘Ruiza 816′). Each plot was spaced 80 cm apart, and rows within plots spaced 60 cm apart. Plot lengths were 2.4 m in 2016, and 3.0 m in 2017 and 2018. A 0.7 m pavement separated the field experiments.

For salt stress treatment, shallow saline groundwater with concentration of 5 g/L (85 mM) saline was used to irrigate the field twice in January and March before sowing. For control treatment, the regular irrigation with non-saline water was performed needed. Field management followed the local standard field practices.

Soil sample collection and component detection

Soil samples were collected from the 0–20 cm and 20–40 cm depth after sowing or before harvest. To cover the experiment area, sampling points were chosen every 15 m from north to south in the experiment field. Three soil samples collected for each sample site were mixed into one sample for soil quality determination. Soil saturated paste extracts (1:2 by weight) were prepared to measure the electric conductivity (EC) and total content of water-soluble salt (ρ) (Rhoades 1996).

The measured sample properties of salinity from EC, and ρ for each sampling points are summarized in Table S1.

Fiber sample preparation and evaluation

Twenty-five naturally opened bolls in the middle of plants were hand-harvested for each plot at crop maturity. A total of 367 and 363 fiber samples were prepared in 2016 on salt stress and normal conditions, respectively. In 2017, 362 and 358 fiber samples were collected from the two conditions. In 2018, a total of 362 fiber samples in each condition were collected. All samples were tested for five fiber quality traits with HVI 900 instrument (USTER_HVISPECTRUM, SPINLAB, USA) at Cotton Fiber Quality Inspection and Test Center of Ministry of Agriculture (Anyang, China). The fiber quality traits obtained were as follows: 2.5% fiber span length (for short fiber length, unit: mm), fiber uniformity (%), fiber strength (cN/tex), fiber elongation (%), and fiber Micronaire (Shang et al. 2015; Ma et al. 2017).

Dataset constitution and data conversion

Three datasets of (1) salt stress condition (E1), (2) normal condition (E2), and (3) the difference values between salt stress and normal conditions (D-value) were used in the present study. The original data of five fiber quality traits were obtained from the trials under E1 and E2, respectively. To ensure the D-value were positive, a constant (C = 10) was added to convert the data prior to statistical analysis.

DNA extraction and marker detection

Genomic DNA was extracted from the parents and RIL plants using CTAB (Cetyltrimethylammonium bromide) method (Paterson et al. 1993). Genomic DNA of the RILs and two parents (GX1135 and GX100-2) was used to construct Illumina libraries with an insert size of 300–400 bp on the Illumina HiSeq platform. The clean reads were aligned to the G. hirsutum accession Texas Marker-1 (TM-1) reference genome using BWA software. The alignment files were converted into BAM files and then sorted using Samtools software (Li et al. 2009). The sorted reads in BAM files used in variant calling. SNP calling on a population scale was performed with the Genome Analysis Toolkit (GATK) (McKenna et al. 2010).

Genetic linkage map construction

Linkage map analysis was conducted using Join Map 4.0. The adjacent markers from the same parent were recorded as one bin (Xie et al. 2010). The linkage map was constructed after the repetition was removed from the markers within the distance of 10 kb. 27,387 SNP (single nucleotide polymorphism), or InDel (insertion or deletion) markers were divided into 26 linkage groups by Logarithm of Odds (LOD) > 9, and 654 SSR markers involved in the original SSR genetic map (Shang et al. 2016b) were selected by LOD > 3. Finally, a total of 330 SSR markers were distributed in the new linkage map. Recombination frequencies were converted into map distances (cM) using the Kosambi mapping function (Kosambi 1944).

The Chi-square test was to determine if the observed genetic segregation ratios of alleles were consistent with expected segregation ratios. A region on the genetic map with at least six adjacent loci showing significant segregation distortion (P < 0.05) was defined as the segregation distortion region (SDR).
Data analysis and QTL mapping

The experimental data were analyzed by the software SPSS (Version 20.0, SPSS, Chicago). QTL mapping and the genetic effect values at single-locus level were conducted by QTL Cartographer software (Version 2.5) using the composite interval mapping (CIM) method (Zeng 1994; Wang et al. 2007). We set parameter in the confidence interval of 95% with composite interval mapping (CIM) method for QTL mapping. The threshold of LOD values were estimated after 1000 permutations tests to declare a significant QTL with a significance level of $P < 0.05$, whereas QTL in another trial with LOD of at least 2.0 was considered as common QTL (Shao et al. 2014). Common QTL were declared according to the position linked and if they shared one or two common markers (Shao et al. 2014).

Candidate gene identification and annotation

The genes located in the confidence intervals of the important QTL were fetched from the Cottongen (https://www.cottongen.org) using their flanking marker positions in G. hirsutum TM-1 genome (Zhang et al. 2015) and considered as candidate genes. Gene ontology (GO) enrichment and KEGG pathway analysis were carried out for all candidate genes. The GO enrichment was performed using GO databases (https://archive.geneontology.org/latest-lite/). To further screen the possible candidate genes involved in fiber development, the gene expression pattern of candidate genes in different period of fiber development was analyzed using the cotton functional genome database (https://www.cottonfgd.org).

RNA extraction and gene expression validation

Total RNA was isolated from 0, 5, 10, 15, and 20 DPA fiber in extremely long fiber length line (H), extremely short fiber length line (L), female (GX1135, F), and male (GX100-2, M). The concentration and purity of total RNA were determined using the Nano Drop spectrophotometry and agarose gel electrophoresis, respectively. RNA samples were stored at $-80 \, ^\circ\mathrm{C}$ freezer for future use.

To validate the potential function in fiber development, the expression patterns of candidate genes were verified with qRT-PCR using RNA of fiber in different development period of extremely lines in fiber length trait and parents of the population. Gene relative expression level was calculated with $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Primers for the qRT-PCR analysis are listed in Table S3. Three independent replicates were performed for each sample. GhUBQ7 gene was used as a reference gene.

Results

Phenotypic performance of fiber quality traits under salt stress and normal conditions

According to the grading standard of saline soil, the average EC of 0–20 cm soil samples collected during three years trial showed moderate salinization (323.11–648.22 μs·cm$^{-1}$) in saline soil and mild salinization (350.08–455.33 μs·cm$^{-1}$) in normal soil, respectively (Table S1, Table S2) (Wang et al. 1993). The average EC of 20–40 cm was moderate salinization (656.78–943.92 μs·cm$^{-1}$) in saline soil and mild salinization (299.22–427.42 μs·cm$^{-1}$), respectively. It is indicated that there was a significant difference in salt concentration between the two areas after saline irrigation (Wang et al. 1993; Table S2).

For the datasets of E1, E2, and D-value, phenotypic performance of fiber quality traits is shown in Tables 1 and 2, including all lines from the RIL population, F1 hybrid ‘Xinza 1’, female parent GX1135, male parent GX100-2, and competitive check hybrid ‘Ruiza 816’ (Shang et al. 2015). The performance of maternal parent GX1135 was often superior to that of male parent GX100-2 whether on E1 or E2. For GX1135 and GX100-2 with homozygous alleles, the trend of phenotypic values on E1 and E2 for fiber length (FL), fiber uniformity (FU) ratio and fiber strength (FS) across three years was not consistent. The phenotypic values on salt stress condition increased much for Micronaire (FM), but had no significant change for fiber elongation (FE) (Table 1). Moreover, mean value of F1 hybrid ‘Xinza 1’ for FM showed no incremental change. It revealed that responding mechanisms to salt stress may differ in the hybrid and its parents (Table 1).

In the RIL population, skewness and kurtosis values of five fiber quality traits ranged between −1.00 and 1.00 for both growing conditions, except that of FU under E1 in 2016 and 2017 (Table 1). Five fiber quality traits showed an abundance of variation in the in the RIL population either on E1 or E2. All fiber quality traits exhibited coefficient of variation less than 15%, indicating that the original data for these traits was reliable. Compared with E2, phenotypic values in the RIL population decreased for FL, FU and FS, but increased for FM and FE under E1 in 2016. In 2017, phenotypic values under E1 were increased for FL, FU, FS, and FE, but decreased for FM. In 2018, phenotypic values under E1 were decreased for FL, FU, and FE, however, increased for FS. These results indicate that the data obtained were credible.
Correlation analysis between fiber quality traits

Table 3 presents correlation analysis on five fiber quality traits using datasets of mean phenotypic values on E1, E2, and D-value.

Under E2, FL showed significant positive correlation with FS, FE, and FU, but was negatively correlated with FM during the three years of this trial. FS showed significant positive correlation with FE, and negative correlation with FM in three years. No significant correlation was observed between FU and FM. The results under E2 showed similar tendency and compared similarly to previous results (Liang et al. 2013; Shang et al. 2015). It revealed that the original data were reasonable for dissecting the genetic basis of fiber quality traits under both growing conditions.

Under E1, FL showed significant positive correlation with FS and FE, but showed negative correlation with FM in 2016, and significant positive correlation with FM in 2017. FU showed significant positively correlation with FE and FM during three years. FS showed significant positively correlation with FE each year, and significant positive correlated with FM in 2017, but was negatively with FM in 2016 and in 2018.
For FM, significant negative correlation existed with FL and FS under E2, but the correlative tendency under E1 was not consistent each year. FU showed significant positive correlation with FL and FS under E2 and showed significant positive correlation or no significant correlation under E1. It revealed that salt stress reduces the correlation between FL and FU, as well as FS and FU. FU showed significant positive correlation with FE and FM over three years, while the correlation between FU and FE, FU, and FM under E2 showed decreased, it indicated that salt stress enhanced the correlation dramatically. Correlations between FE and FM were not significant under E1 and E2. FE showed positive significant correlation with FS under both conditions. It indicated that salt stress had no effect on the correlations between FE and FS, FE, and FM.

Table 2  Descriptive statistical analysis for fiber quality traits on the difference value dataset between salt stress condition and normal condition

| Traits   | Year | Mean | SD  | CV% | Min  | Max  | Skewness | Kurtosis | GX1135 | GX100-2 | Xinza1 | Ruiza 816 |
|----------|------|------|-----|-----|------|------|----------|----------|--------|---------|--------|----------|
| FL (mm)  | 2016 | 9.99 | 0.95| 9.53| 4.95 | 12.70| −0.57    | 4.26     | 9.80   | 9.80    | 9.60   | 9.95     |
|          | 2017 | 10.44| 0.98| 9.39| 7.85 | 12.90| −0.10    | −0.41    | 11.30  | 11.25   | 11.10  | 9.75     |
|          | 2018 | 9.60 | 0.84| 8.79| 7.55 | 12.35| 0.00     | 0.02     | 9.00   | 9.50    | 7.95   | 10.30    |
| FU (%)   | 2016 | 9.76 | 0.97| 9.97| 5.40 | 12.70| −0.60    | 2.54     | 9.33   | 9.75    | 8.82   | 10.05    |
|          | 2017 | 10.79| 1.00| 9.23| 7.45 | 13.20| −0.28    | 0.23     | 9.50   | 10.70   | 9.50   | 9.75     |
|          | 2018 | 8.78 | 1.29| 14.69|5.55 | 12.20| 0.09     | −0.18    | 8.85   | 10.15   | 7.20   | 9.75     |
| FS (cN/tex) | 2016 | 8.87 | 1.21| 13.64|5.00 | 12.65| 0.03     | 0.96     | 10.02  | 8.15    | 8.65   | 9.28     |
|          | 2017 | 10.90| 1.52| 13.96|6.65 | 15.70| 0.14     | −0.11    | 8.45   | 12.60   | 11.55  | 9.15     |
|          | 2018 | 11.01| 1.66| 15.07|6.00 | 15.30| −0.34    | −0.03    | 10.50  | 11.45   | 7.55   | 12.15    |
| FE (%)   | 2016 | 10.02| 0.08| 0.82| 9.70 | 10.35| 0.37     | 2.34     | 10.02  | 9.97    | 9.95   | 9.97     |
|          | 2017 | 10.07| 0.08| 0.84| 9.85 | 10.35| 0.11     | 0.14     | 10.00  | 10.00   | 10.00  | 10.00    |
|          | 2018 | 10.00| 0.05| 0.55| 9.80 | 10.15| −0.50    | 1.22     | 10.10  | 10.10   | 9.75   | 10.00    |
| FM       | 2016 | 10.12| 0.40| 3.97| 8.50 | 11.15| −0.19    | 1.09     | 10.20  | 10.55   | 9.75   | 9.93     |
|          | 2017 | 9.96 | 0.19| 1.95| 9.20 | 10.70| −0.15    | 2.45     | 10.25  | 10.05   | 9.70   | 10.10    |
|          | 2018 | 9.91 | 0.20| 2.00| 9.45 | 10.50| 0.30     | 0.11     | 10.00  | 10.00   | 9.80   | 10.05    |

Construction of genetic map

Among the 174,351 markers, the low-coverage sequences of the RIL populations were filtered out, leaving 34,361 markers. After filtering SNPs according to the genotyping criteria, a total of 27,387 homozygous markers were identified between the two parents to generate bin markers for the RIL population. Finally, a total of 27,387 polymorphic SNP or Indel markers and 330 SSR markers were used for the construction of linkage map. Adjacent markers with the same genotype were merged as an identical bin. A high-density genetic linkage map was constructed with 2859 recombination bin markers. Distribution of markers and marker interval on chromosomes based on RIL linkage map is shown in Table 4. The genetic map covered 2133.53 cM of cotton genome with average interval of 0.785 cM. Among the 26 linkage groups, the average bin interval was 5.979 cM, and the range of interval on 26 chromosomes was 3.647–10.361 cM, and only one gap larger than 10 cM was observed in chromosome D05. The length of the linkage groups ranged from 34.181 cM (A09) to 149.966 cM (A05). The linkage map harboring these markers is shown in Table S4 and Fig. S1. On an average, one linkage group harbored about 110 bins that covered an average of 82.059 cM. The average bin interval was 0.758 cM with a range of 0.523 cM (A13) to 0.939 cM (D01). The highest number of markers (181) were present in the chromosome A05 with an average marker interval of 1.207 cM.

Segregation distortion

Among the 2859 polymorphic loci, 336 (11.75%) showed segregation distortion ($P < 0.05$) with 213 (63.39%) favoring the GX1135 alleles and 123 (36.61%) favoring the GX100-2 alleles (Table S5). These distorted loci existed on 19 chromosomes and mapped unevenly on different chromosomes. More distorted loci were located on Dt subgenome than on At subgenome (142 versus 142). Among them, Chr. D01 and D04 accounted for 61.36% and 51.32% of distorted loci on corresponding chromosome, respectively (Table S5). There are 280 (83.33%) of the 336 distorted loci were clustered into 15 SDRs, with nine located on At subgenome and six on Dt subgenome. Chr. D11 was heavily concentrated with distorted loci, with 51 loci showing significant distortion toward GX100-2, forming a large SDR of 40.451 cM (Table S6). SDR12 which located on Chr. D04 showing significant distortion toward GX1135 and forming the largest interval of 40.561 cM (Table S6).
QTL mapping for fiber quality

A total of 159 QTL controlling fiber quality traits were detected under E1 and E2, explaining 2.71–16.03% of total phenotypic variance (PV) (Table 5). Separately, 51, 70, and 53 QTL were detected in the RIL population under E1, E2, and D-value. Twelve QTL were detected in two or three years, of which seven, one, four, one, and one QTL were detected for FL, FU, FS, FE, and FM, respectively. A total of 10 QTL were identified in at least two datasets, three QTL were detected in E1 and E2 for FL, and one QTL was detected on E1 and D-value for FS. For FM, six QTL were detected on two datasets: three QTL on E1 and E2, two QTL on E2 and D-value, one QTL on E1 and D-value. However, no QTL were detected for FE and FU.
For FL, a total of 40 QTL located on 19 chromosomes were identified. Of those, 14 QTL was detected under E1, 17 and 11 QTL were identified in RIL population under E1 and D-value, respectively (Table 5). Seven QTL detected on E1 increased FL with threshold of LOD 2.62 on average. Ten QTL decreased FL explained 6.53% of PV on average. The qFL-Chr1-1, qFL-Chr5-5, qFL-Chr24-4, which were detected on both E1 and E2, explained 3.68, 11.65, and 4.09% of PV, respectively. Three QTL were mapped with LOD threshold 2.27, 6.72, and 3.87, respectively. Two of these QTL were identified in at least two years.

Out of 26 QTL (LOD ranged from 2.04 to 4.29), 4, 13, and 9 QTL for FU was identified on E1, E2, and D-value, respectively. Four QTL were identified in more than two years, but no QTL were detected in more than two growing conditions in the same year.

For FS, a total of 28 QTL were resolved explaining from 3.66 to 12.90% of PV, among which seven, 12, and 13 QTL were identified on E1, E2, and D-value, respectively. One QTL (qFS-Chr25-2) by the threshold of LOD 5.79 was detected on both E1 and D-value. Two of three QTL located on Chr 24 explained PV more than 10%.

Nine, 15, and five QTL for FE distributed on 18 chromosomes were identified under E1, E2, and D-value, respectively. The stable QTL qFE-Chr20-3, identified in the D-value, was also identified on E2 in 2016 and 2017, with the threshold of LOD 3.44 on average. The QTL, qFE-Chr7-1, was identified under E2 and D-value with the threshold of LOD 4.31 on average. Identified in both E1 and D-value, qFE-Chr15-1 exhibited average LOD of 2.31.

A total of 12, 18, and 8 QTL underlying FM were identified on 16 chromosomes under E1, E2, and D-value, respectively. Of these QTL, qFM-Chr8-3, qFM-Chr15-1, qFM-Chr15-3, and qFM-Chr21-1 were detected repeatedly across two or three years. Three QTL detected on E1 also played important roles in controlling FM on E2, including qFM-Chr5-2, qFM-Chr8-2, and qFM-Chr8-3. Another two QTL were detected on E2 and D-value; these were qFM-Chr15-1 and qFM-Chr16-1. One QTL, qFM-Chr25-1, explained 4.60% of PV and was identified under E1 and D-value.

### Table 4 Distribution of markers and marker interval on chromosomes based on RIL linkage map

| Chr  | Length(cM) | No. of markers | No. of bins | Marker interval (cM) | Bin interval (cM) | Max interval (cM) |
|------|------------|----------------|-------------|---------------------|------------------|------------------|
| A01  | 72.378     | 1646           | 109         | 0.044               | 0.670            | 6.410            |
| A02  | 63.522     | 1115           | 98          | 0.057               | 0.655            | 3.979            |
| A03  | 107.311    | 810            | 142         | 0.133               | 0.761            | 4.311            |
| A04  | 76.875     | 1199           | 138         | 0.064               | 0.561            | 6.410            |
| A05  | 149.966    | 960            | 181         | 0.156               | 0.833            | 5.690            |
| A06  | 65.590     | 3618           | 104         | 0.018               | 0.637            | 3.979            |
| A07  | 106.389    | 1021           | 129         | 0.104               | 0.831            | 6.050            |
| A08  | 72.144     | 476            | 87          | 0.152               | 0.839            | 4.648            |
| A09  | 34.181     | 583            | 45          | 0.059               | 0.777            | 4.991            |
| A10  | 83.374     | 1170           | 107         | 0.071               | 0.787            | 5.365            |
| A11  | 89.789     | 1783           | 129         | 0.050               | 0.701            | 5.690            |
| A12  | 64.627     | 1094           | 104         | 0.059               | 0.627            | 3.647            |
| A13  | 54.408     | 2366           | 105         | 0.023               | 0.523            | 3.647            |
| D01  | 81.679     | 588            | 88          | 0.139               | 0.939            | 7.153            |
| D02  | 103.223    | 1266           | 145         | 0.082               | 0.717            | 7.154            |
| D03  | 87.538     | 1191           | 115         | 0.074               | 0.768            | 3.647            |
| D04  | 69.669     | 326            | 76          | 0.214               | 0.929            | 6.781            |
| D05  | 84.896     | 636            | 92          | 0.134               | 0.933            | 10.361           |
| D06  | 85.986     | 787            | 124         | 0.109               | 0.699            | 5.340            |
| D07  | 117.105    | 1227           | 153         | 0.096               | 0.770            | 6.049            |
| D08  | 89.787     | 1078           | 120         | 0.083               | 0.755            | 6.050            |
| D09  | 78.327     | 398            | 88          | 0.197               | 0.900            | 8.306            |
| D10  | 65.318     | 394            | 78          | 0.166               | 0.848            | 8.305            |
| D11  | 94.903     | 507            | 113         | 0.188               | 0.847            | 8.305            |
| D12  | 87.626     | 558            | 113         | 0.157               | 0.782            | 6.410            |
| D13  | 46.917     | 590            | 76          | 0.080               | 0.626            | 6.781            |
| Whole| 2133.528   | 27,387         | 2859        | 0.104               | 0.758            | 5.979            |
| Trait | QTL       | Year | Flanking markers | Under E1 |           | Under E2 |           | Mapping by D-value |
|-------|-----------|------|------------------|----------|-----------|----------|-----------|-------------------|
|       |           |      |                  | LOD      | Effect    | Var%     | LOD       | Effect    | Var%     |
|       | FL        | 2016 | Bin28 Bin29      | 2.01     | 0.22      | 3.31     | 2.27      | 0.23      | 3.68     |
|       |           | 2018 | Bin48 Bin49      |          |           |          | 2.90      | 0.28      | 5.83     |
|       | qFL-Chr4-1| 2016 | Bin400 Bin401    | 2.44     | 0.24      | 4.20     |           |           |          |
|       | qFL-Chr4-2| 2016 | Bin421 Bin422    | 2.48     | 0.24      | 4.06     |           |           |          |
|       | qFL-Chr5-1| 2016 | Bin490 Bin491    |          |           |          | 4.61      | 0.34      | 8.20     |
|       |           | 2017 | Bin493 Bin494    | 6.49     | 0.40      | 12.11    |           |           |          |
|       | qFL-Chr5-2| 2016 | Bin500 Bin501    | 2.79     | 0.26      | 4.87     |           |           |          |
|       |           | 2017 | Bin502 Bin503    | 5.08     | 0.36      | 9.66     |           |           |          |
|       | qFL-Chr5-3| 2016 | Bin512 Bin513    |          |           |          | 5.87      | 0.37      | 10.31    |
|       |           | 2017 | Bin515 Bin516    | 6.23     | 0.36      | 11.06    |           |           |          |
|       | qFL-Chr5-4| 2017 | Bin527 Bin528    | 6.90     | 0.38      | 12.14    |           |           |          |
|       | qFL-Chr5-5*| 2016 | Bin648 Bin649    | 3.29     | 0.26      | 5.68     |           |           |          |
|       |           | 2016 | Bin648 Bin649    | 5.91     | 0.39      | 10.80    | 6.73      | 0.40      | 11.65    |
|       | qFL-Chr6-1| 2016 | Bin760 Bin761    | 3.84     | 0.31      | 6.82     |           |           |          |
|       | qFL-Chr7-1| 2016 | Bin795 Bin796    | 2.49     | 0.23      | 5.57     |           |           |          |
|       | qFL-Chr7-2| 2016 | Bin837 Bin838    | 2.30     | 0.22      | 4.85     |           |           |          |
|       | qFL-Chr7-3| 2017 | Bin844 Bin845    | 3.34     | 0.28      | 6.35     |           |           |          |
|       | qFL-Chr7-4| 2017 | Bin851 Bin852    | 2.30     | 0.22      | 4.02     |           |           |          |
|       | qFL-Chr9-1| 2018 | Bin1024 Bin1025  | 2.35     | 0.24      | 4.70     |           |           |          |
|       | qFL-Chr11-1| 2017 | Bin1168 Bin1169  | 2.23     | 0.21      | 4.48     |           |           |          |
|       | qFL-Chr12-1| 2018 | Bin1368 Bin1369  | 2.97     | 0.22      | 6.17     |           |           |          |
|       |           | 2018 | Bin1371 Bin1372  | 2.72     | 0.27      | 5.45     |           |           |          |
|       | qFL-Chr13-1| 2017 | Bin1381 Bin1382  | 3.67     | 0.30      | 6.60     |           |           |          |
|       | qFL-Chr13-2| 2017 | Bin1389 Bin1390  | 3.26     | 0.29      | 6.14     |           |           |          |
|       | qFL-Chr14-1| 2018 | Bin1483 Bin1484  | 2.33     | 0.24      | 4.64     |           |           |          |
|       | qFL-Chr14-2| 2018 | Bin1517 Bin1518  | 2.13     | 0.23      | 4.01     |           |           |          |
|       | qFL-Chr14-3| 2016 | Bin1529 Bin1530  | 2.13     | 0.21      | 4.40     |           |           |          |
|       | qFL-Chr15-1| 2017 | Bin1599 Bin1600  | 2.47     | 0.23      | 5.15     |           |           |          |
|       |           | 2017 | Bin1694 Bin1695  | 2.31     | 0.22      | 3.71     |           |           |          |
|       | qFL-Chr16-1| 2016 | Bin1707 Bin1708  | 2.02     | 0.23      | 4.00     |           |           |          |
|       | qFL-Chr16-2| 2017 | Bin1784 Bin1785  | 2.10     | 0.20      | 3.38     |           |           |          |
|       | qFL-Chr18-1| 2017 | Bin1933 Bin1934  | 2.07     | 0.21      | 3.33     |           |           |          |
|       | qFL-Chr19-1| 2017 | Bin1996 Bin1997  | 2.38     | 0.24      | 4.21     |           |           |          |
|       | qFL-Chr21-1| 2017 | Bin2335 Bin2336  | 2.84     | 0.24      | 4.87     |           |           |          |
|       | qFL-Chr21-2| 2016 | Bin2338 Bin2339  | 3.19     | 0.28      | 5.65     |           |           |          |
|       |           | 2016 | Bin2347 Bin2348  | 2.25     | 0.24      | 4.10     |           |           |          |
Table 5 (continued)

| Trait QTL | Year | Flanking markers | Under $E_1$ | Under $E_2$ | Mapping by $D$-value |
|-----------|------|------------------|-------------|-------------|----------------------|
|           |      |                  | LOD         | Effect value | Var%                 |
|           |      |                  |             |              |                      |
| $qFL$-Chr23-1 | 2018 | Bin2499 Bin2500  | 3.22        | 0.22        | 6.72                 |
| $qFL$-Chr23-1 | 2018 | Bin2503 Bin2504  | 2.11        | 0.24        | 4.73                 |
| $qFL$-Chr23-2 | 2018 | Bin2512 Bin2522  | 2.83        | -0.33       | 5.93                 |
| $qFL$-Chr24-1 | 2017 | Bin2558 Bin2559  | 2.67        | 0.26        | 4.74                 |
| $qFL$-Chr24-2 | 2016 | Bin2581 Bin2582  | 2.66        | 0.25        | 4.52                 |
| $qFL$-Chr24-2 | 2016 | Bin2587 Bin2588  | 2.61        | 0.26        | 4.67                 |
| $qFL$-Chr24-3 | 2017 | Bin2593 Bin2594  | 4.08        | 0.31        | 6.80                 |
| $qFL$-Chr24-4 | 2016 | Bin2606 Bin2607  | 2.27        | 0.25        | 4.09                 |
| $qFL$-Chr24-5 | 2017 | Bin2627 Bin2628  | 2.10        | 0.22        | 4.47                 |
| $qFL$-Chr25-1 | 2017 | Bin2674 Bin2675  | 2.07        | -0.21       | 4.35                 |
| $qFL$-Chr25-2 | 2017 | Bin2683 Bin2684  | 4.62        | -0.31       | 10.09                |
| $qFL$-Chr25-3 | 2017 | Bin2690 Bin2691  | 4.22        | -0.29       | 8.61                 |
| $qFU$-Chr2-1 | 2018 | Bin110 Bin111    | 2.88        | -0.33       | 6.12                 |
| $qFU$-Chr4-1 | 2017 | Bin472 Bin473    | 3.10        | -0.26       | 6.48                 |
| $qFU$-Chr5-1 | 2017 | Bin492 Bin493    | 2.66        | -0.20       | 5.18                 |
| $qFU$-Chr5-2 | 2016 | Bin554 Bin555    | 2.45        | 0.22        | 4.99                 |
| $qFU$-Chr6-1 | 2016 | Bin721 Bin722    | 2.09        | -0.15       | 4.21                 |
| $qFU$-Chr7-1 | 2017 | Bin775 Bin776    | 2.04        | -0.17       | 3.79                 |
| $qFU$-Chr7-2 | 2018 | Bin840 Bin841    | 2.20        | -0.18       | 4.39                 |
| $qFU$-Chr7-3 | 2018 | Bin857 Bin858    | 2.24        | -0.20       | 4.62                 |
| $qFU$-Chr7-4 | 2018 | Bin867 Bin868    | 2.22        | -0.20       | 4.59                 |
| $qFU$-Chr9-1 | 2016 | Bin991 Bin992    | 3.62        | -0.20       | 6.99                 |
| $qFU$-Chr11-1 | 2016 | Bin1229 Bin1230  | 2.09        | -0.15       | 4.10                 |
| $qFU$-Chr13-1 | 2016 | Bin1374 Bin1375  | 3.49        | -0.19       | 6.72                 |
| $qFU$-Chr13-2 | 2017 | Bin1499 Bin1500  | 4.29        | 0.22        | 8.24                 |
| $qFU$-Chr13-3 | 2018 | Bin1467 Bin1468  | 2.15        | 0.30        | 4.54                 |
| $qFU$-Chr14-1 | 2018 | Bin1520 Bin1521  | 3.52        | 0.26        | 8.16                 |
| $qFU$-Chr15-1 | 2016 | Bin1579 Bin1580  | 2.97        | -0.18       | 5.70                 |
| $qFU$-Chr15-2 | 2016 | Bin1599 Bin1600  | 2.65        | 0.23        | 5.54                 |
| $qFU$-Chr15-3 | 2017 | Bin1620 Bin1621  | 2.25        | 0.19        | 4.84                 |
| $qFU$-Chr18-1 | 2016 | Bin1904 Bin1905  | 3.64        | 0.24        | 7.70                 |
| $qFU$-Chr19-1 | 2017 | Bin2057 Bin2058  | 2.45        | 0.20        | 5.13                 |
| $qFU$-Chr23-1 | 2017 | Bin2554 Bin2555  | 2.05        | 0.21        | 4.38                 |
| $qFU$-Chr24-1 | 2016 | Bin2558 Bin2559  | 3.37        | -0.30       | 6.93                 |
| $qFU$-Chr24-2 | 2016 | Bin2586 Bin2587  | 2.23        | 0.24        | 4.52                 |
| $qFU$-Chr24-3 | 2017 | Bin2630 Bin2631  | 2.74        | 0.19        | 5.78                 |
| $qFU$-Chr25-1 | 2016 | Bin2681 Bin2682  | 2.20        | 0.18        | 4.54                 |
| $qFU$-Chr25-2 | 2018 | Bin2763 Bin2764  | 2.70        | -0.31       | 5.72                 |
| $qFS$-Chr1-1 | 2018 | Bin3 Bin4        | 2.92        | 0.45        | 6.21                 |
| Trait QTL      | Year | Flanking markers | Under E1 | Mapping by D-value | Under E2 | Var%b | LOD | Effect value | Var% | LOD | Effect value | Var% |
|---------------|------|------------------|---------|-------------------|---------|-------|-----|--------------|-----|-----|--------------|-----|
| qFS-Chr2-1    | 2017 | Bin139           | Bin140  |                   | 2.26    | 0.32  | 4.11|
| qFS-Chr5-1    | 2016 | Bin488           | Bin489  |                   | 3.60    | 0.31  | 6.50|
| qFS-Chr5-2    | 2016 | Bin500           | Bin501  |                   | 2.31    | 0.25  | 4.24|
| qFS-Chr5-3    | 2017 | Bin502           | Bin503  |                   | 2.31    | 0.25  | 4.04|
| qFS-Chr5-4    | 2016 | Bin638           | Bin639  |                   | 2.43    | 0.34  | 4.90|
| qFS-Chr5-5    | 2016 | Bin638           | Bin639  |                   | 2.98    | 0.29  | 5.61|
| qFS-Chr5-6    | 2017 | Bin640           | Bin641  |                   | 3.98    | 0.45  | 7.06|
| qFS-Chr5-7    | 2016 | Bin638           | Bin639  |                   | 2.31    | 0.25  | 4.04|
| qFS-Chr7-1    | 2017 | Bin789           | Bin790  |                   | 2.36    | 0.31  | 4.35|
| qFS-Chr7-2    | 2017 | Bin804           | Bin805  |                   | 2.98    | 0.37  | 5.82|
| qFS-Chr7-3    | 2018 | Bin868           | Bin869  |                   | 2.27    | 0.41  | 5.11|
| qFS-Chr8-1    | 2017 | Bin912           | Bin913  |                   | 2.27    | 0.37  | 4.85|
| qFS-Chr8-2    | 2017 | Bin872           | Bin873  |                   | 2.75    | 0.38  | 5.00|
| qFS-Chr11-1   | 2017 | Bin1010          | Bin1011 |                   | 3.18    | 0.02  | 6.10|
| qFS-Chr14-1   | 2016 | Bin1501          | Bin1502 |                   | 3.08    | 0.30  | 5.42|
| qFS-Chr15-1   | 2017 | Bin1659          | Bin1660 |                   | 2.14    | 0.29  | 3.73|
| qFS-Chr17-1   | 2017 | Bin1861          | Bin1862 |                   | 2.95    | 0.44  | 5.75|
| qFS-Chr17-2   | 2016 | Bin1870          | Bin1871 |                   | 2.06    | 0.29  | 4.36|
| qFS-Chr18-1   | 2017 | Bin1909          | Bin1910 |                   | 3.33    | 0.39  | 6.51|
| qFS-Chr19-1   | 2016 | Bin2066          | Bin2067 |                   | 2.75    | 0.36  | 5.35|
| qFS-Chr22-1   | 2017 | Bin2411          | Bin2412 |                   | 2.72    | 0.52  | 6.64|
| qFS-Chr24-1   | 2017 | Bin2578          | Bin2579 |                   | 6.25    | 0.56  | 11.19|
| qFS-Chr24-2   | 2017 | Bin2581          | Bin2582 |                   | 2.90    | 0.35  | 5.39|
| qFS-Chr24-3   | 2017 | Bin2584          | Bin2585 |                   | 2.60    | 0.27  | 4.66|
| qFS-Chr24-2   | 2016 | Bin2593          | Bin2594 |                   | 2.74    | 0.34  | 5.10|
| qFS-Chr25-1   | 2016 | Bin2596          | Bin2597 |                   | 2.74    | 0.34  | 5.10|
| qFS-Chr25-2   | 2017 | Bin2601          | Bin2602 |                   | 4.78    | 0.51  | 8.72|
| qFS-Chr25-2   | 2017 | Bin2707          | Bin2708 |                   | 2.03    | 0.24  | 3.66|
| qFS-Chr25-2   | 2018 | Bin2712          | Bin2713 |                   | 2.67    | 0.28  | 4.78|
| qFS-Chr25-2   | 2017 | Bin2769          | Bin2770 |                   | 2.17    | 0.27  | 4.95|
| FE            |      |                  |         |                   | 2.95    | 0.02  | 6.44|
| qFE-Chr2-1    | 2017 | Bin128           | Bin129  |                   | 5.49    | 0.03  | 11.13|
| qFE-Chr3-1    | 2016 | Bin493           | Bin494  |                   | 3.26    | 0.02  | 6.42|
| qFE-Chr3-1    | 2016 | Bin521           | Bin522  |                   | 3.26    | 0.02  | 6.42|
| qFE-Chr3-1    | 2016 | Bin530           | Bin531  |                   | 2.13    | 0.02  | 4.36|
| qFE-Chr3-1    | 2018 | Bin872           | Bin873  |                   | 3.33    | 0.01  | 6.80|
| qFE-Chr3-1    | 2018 | Bin879           | Bin880  |                   | 5.28    | 0.02  | 11.19|
| qFE-Chr9-1    | 2017 | Bin1010          | Bin1011 |                   | 3.18    | 0.02  | 6.10|
| qFE-Chr10-1   | 2017 | Bin1123          | Bin1124 |                   | 3.04    | 0.02  | 5.98|
| qFE-Chr10-1   | 2017 | Bin1132          | Bin1133 |                   | 3.72    | 0.02  | 7.44|
Table 5 (continued)

| Trait | QTL | Year | Flanking markers | Under E1<sup>c</sup> | Under E2 | Mapping by D-value |
|-------|-----|------|------------------|---------------------|----------|---------------------|
|       |     |      |                  | LOD     | Effect value | Var%<sup>b</sup> | LOD     | Effect value | Var%  |
|       |     |      |                  |         |             |            |         |             |       |
| qFE-Chr11-1 | 2018 | Bin1192 | Bin1193 | 3.16 | – 0.01 | 6.43 |
| qFE-Chr12-1 | 2016 | Bin1364 | Bin1365 | 2.08 | 0.02 | 4.65 |
| qFE-Chr14-1 | 2018 | Bin1494 | Bin1495 | 3.65 | – 0.01 | 7.66 |
| qFE-Chr14-2 | 2018 | Bin1508 | Bin1509 | 4.60 | – 0.01 | 9.54 |
| qFE-Chr14-3 | 2018 | Bin1516 | Bin1517 | 3.17 | – 0.01 | 6.68 |
| qFE-Chr15-1 | 2016 | Bin1589 | Bin1590 | 2.04 | 0.02 | 4.52 |
|       | 2016 | Bin1596 | Bin1597 | 2.80 | 0.02 | 5.68 |
|       | 2016 | Bin1599 | Bin1600 | 2.08 | 0.02 | 4.22 |
| qFE-Chr16-1 | 2016 | Bin1718 | Bin1719 | 2.94 | – 0.02 | 5.94 |
| qFE-Chr17-1 | 2017 | Bin1835 | Bin1836 | 2.17 | 0.02 | 4.35 |
| qFE-Chr18-1 | 2018 | Bin1971 | Bin1972 | 3.04 | – 0.01 | 6.19 |
| qFE-Chr19-1 | 2018 | Bin2089 | Bin2090 | 2.42 | – 0.01 | 4.87 |
| qFE-Chr19-2 | 2018 | Bin2099 | Bin2100 | 2.37 | – 0.01 | 4.78 |
| qFE-Chr20-1 | 2017 | Bin2185 | Bin2186 | 3.76 | – 0.02 | 7.13 |
| qFE-Chr20-2 | 2018 | Bin2204 | Bin2204 | 2.05 | 0.01 | 3.90 |
| qFE-Chr20-3 | 2016 | Bin2248 | Bin2249 | 4.64 | 0.03 | 10.43 |
|       | 2016 | Bin2254 | Bin2255 | 3.62 | 0.02 | 7.41 |
|       | 2017 | Bin2267 | Bin2268 | 2.07 | 0.01 | 3.63 |
| qFE-Chr21-1 | 2018 | Bin2279 | Bin2280 | 4.21 | – 0.02 | 8.83 |
| qFE-Chr21-2 | 2017 | Bin2336 | Bin2337 | 2.56 | 0.02 | 4.76 |
| qFE-Chr21-3 | 2017 | Bin2345 | Bin2346 | 2.28 | 0.02 | 4.37 |
| qFE-Chr22-1 | 2017 | Bin2393 | Bin2394 | 2.90 | 0.02 | 5.96 |
| qFE-Chr22-2 | 2017 | Bin2412 | Bin2413 | 2.55 | 0.02 | 4.44 |
| qFE-Chr24-1 | 2018 | Bin2584 | Bin2585 | 3.70 | 0.01 | 7.59 |
| qFE-Chr25-1 | 2017 | Bin2695 | Bin2696 | 2.90 | – 0.02 | 5.95 |
| FM     | qFM-Chr1-1 | 2016 | Bin96 | Bin97 | 3.13 | 0.10 | 6.03 |
|       | qFM-Chr2-1 | 2017 | Bin128 | Bin129 | 2.71 | – 0.06 | 4.02 |
|       | qFM-Chr4-1 | 2018 | Bin360 | Bin361 | 2.75 | 0.08 | 5.74 |
|       | qFM-Chr5-1 | 2018 | Bin366 | Bin367 | 2.18 | 0.07 | 4.58 |
|       | qFM-Chr5-2 | 2017 | Bin484 | Bin485 | 3.99 | – 0.07 | 5.72 |
|       | qFM-Chr5-3 | 2017 | Bin488 | Bin489 | 2.21 | 0.05 | 2.91 |
|       | qFM-Chr5-4 | 2017 | Bin641 | Bin642 | 6.00 | 0.09 | 9.37 |
|       | qFM-Chr8-1 | 2018 | Bin645 | Bin646 | 4.85 | 0.09 | 7.98 |
|       | qFM-Chr8-2 | 2018 | Bin655 | Bin656 | 6.06 | 0.10 | 9.81 |
|       | qFM-Chr8-3 | 2017 | Bin657 | Bin658 | 7.24 | 0.10 | 11.13 |
|       | qFM-Chr8-4 | 2016 | Bin842 | Bin843 | 2.53 | 0.08 | 4.51 |
|       | qFM-Chr8-5 | 2016 | Bin850 | Bin851 | 2.16 | 0.08 | 3.95 |
|       | qFM-Chr8-6 | 2018 | Bin910 | Bin911 | 2.84 | 0.08 | 5.85 |
|       | qFM-Chr8-7 | 2018 | Bin919 | Bin920 | 2.99 | 0.08 | 6.02 |
Table 5 (continued)

| Trait QTL   | Year | Flanking markers | Under E1<sup>c</sup> LOD | Effect value | Var%<sup>b</sup> | Under E2 LOD | Effect value | Var% | Mapping by D-value LOD | Effect value | Var% |
|-------------|------|------------------|---------------------------|--------------|----------------|----------------|--------------|------|------------------------|--------------|------|
| qFM-Chr8-3* | 2017 | Bin964 Bin965    | 2.73                      | 0.06         | 4.04           |                |              |      |                        |              |      |
|             | 2017 | Bin977 Bin978    | 2.02                      | 0.06         | 3.01           |                |              |      |                        |              |      |
|             | 2016 | Bin969 Bin970    | 3.48                      | 0.10         | 6.71           |                |              |      |                        |              |      |
|             | 2018 | Bin977 Bin978    | 2.34                      | 0.07         | 4.85           | 3.46           | 0.09         | 7.30 |                        |              |      |
|             | 2018 | Bin982 Bin983    | 2.84                      | 0.07         | 5.86           |                |              |      |                        |              |      |
| qFM-Chr13-1 | 2018 | Bin1434 Bin1435  | 2.49                      | 0.05         | 5.41           |                |              |      |                        |              |      |
| qFM-Chr13-2 | 2018 | Bin1456 Bin1457  | 2.03                      | 0.04         | 4.44           |                |              |      |                        |              |      |
| qFM-Chr13-3 | 2016 | Bin1474          | 2.20                      | 0.09         | 3.84           |                |              |      |                        |              |      |
| qFM-Chr14-1 | 2017 | Bin1502 Bin1503  | 3.11                      | 0.07         | 4.59           |                |              |      |                        |              |      |
| qFM-Chr14-2 | 2016 | Bin1521 Bin1522  | 2.54                      | 0.10         | 5.57           |                |              |      |                        |              |      |
|             | 2016 | Bin1528 Bin1529  | 2.37                      | 0.09         | 4.99           |                |              |      |                        |              |      |
| qFM-Chr15-1 | 2016 | Bin1568 Bin1569  | 2.08                      | 0.08         | 4.11           |                |              |      |                        |              |      |
|             | 2016 | Bin1579 Bin1580  | 3.84                      | 0.11         | 7.42           |                |              |      |                        |              |      |
|             | 2016 | Bin1580 Bin1581  | 3.38                      | 0.11         | 7.22           |                |              |      |                        |              |      |
|             | 2016 | Bin1589 Bin1590  | 2.48                      | 0.09         | 4.88           | 2.42           | 0.10         | 6.45 |                        |              |      |
|             | 2018 | Bin1571 Bin1572  | 3.55                      | 0.06         | 7.37           |                |              |      |                        |              |      |
|             | 2018 | Bin1594 Bin1595  | 4.94                      | 0.07         | 10.49          |                |              |      |                        |              |      |
| qFM-Chr15-2 | 2017 | Bin1678 Bin1679  | 2.02                      | 0.05         | 2.84           |                |              |      |                        |              |      |
| qFM-Chr15-3 | 2016 | Bin1690 Bin1691  | 3.48                      | 0.10         | 6.72           |                |              |      |                        |              |      |
|             | 2017 | Bin1690 Bin1691  | 2.48                      | 0.06         | 3.84           |                |              |      |                        |              |      |
|             | 2017 | Bin1689 Bin1690  | 5.53                      | 0.10         | 9.13           |                |              |      |                        |              |      |
| qFM-Chr16-1 | 2016 | Bin1817 Bin1818  | 2.03                      | 0.09         | 4.38           |                |              |      |                        |              |      |
|             | 2016 | Bin1821 Bin1822  | 2.11                      | 0.08         | 3.92           | 3.10           | 0.11         | 6.59 |                        |              |      |
| qFM-Chr19-1 | 2017 | Bin2061 Bin2062  | 3.00                      | 0.05         | 6.15           |                |              |      |                        |              |      |
| qFM-Chr19-2 | 2017 | Bin2073 Bin2074  | 2.37                      | 0.04         | 4.88           |                |              |      |                        |              |      |
| qFM-Chr21-1 | 2017 | Bin2279 Bin2280  | 2.05                      | 0.05         | 2.71           |                |              |      |                        |              |      |
|             | 2016 | Bin2285 Bin2286  | 2.00                      | 0.08         | 3.77           |                |              |      |                        |              |      |
|             | 2016 | Bin2292 Bin2293  | 2.77                      | 0.09         | 5.15           |                |              |      |                        |              |      |
| qFM-Chr21-2 | 2017 | Bin2330 Bin2331  | 2.75                      | 0.06         | 5.77           |                |              |      |                        |              |      |
| qFM-Chr22-1 | 2017 | Bin2464 Bin2465  | 3.11                      | 0.07         | 4.97           |                |              |      |                        |              |      |
| qFM-Chr22-2 | 2017 | Bin2472 Bin2473  | 10.29                     | 0.11         | 16.03          |                |              |      |                        |              |      |
|             | 2017 | Bin2473 Bin2474  | 2.44                      | 0.06         | 3.94           |                |              |      |                        |              |      |
| qFM-Chr23-1 | 2017 | Bin2537 Bin2538  | 2.06                      | 0.05         | 2.73           |                |              |      |                        |              |      |
| qFM-Chr25-1 | 2016 | Bin2700 Bin2701  | 2.48                      | 0.09         | 4.91           | 2.06           | 0.08         | 4.32 |                        |              |      |
|             | 2016 | Bin2706 Bin2707  | 2.47                      | 0.08         | 4.56           |                |              |      |                        |              |      |
| qFM-Chr25-2 | 2018 | Bin2726 Bin2727  | 2.07                      | 0.07         | 4.42           |                |              |      |                        |              |      |
| qFM-Chr25-3 | 2018 | Bin2738 Bin2739  | 3.29                      | 0.09         | 6.92           |                |              |      |                        |              |      |
| qFM-Chr25-4 | 2018 | Bin2751 Bin2752  | 2.05                      | 0.07         | 4.39           |                |              |      |                        |              |      |
| qFM-Chr26-1 | 2016 | Bin2810 Bin2811  | 2.37                      | 0.08         | 4.52           |                |              |      |                        |              |      |
| qFM-Chr26-2 | 2016 | Bin2837 Bin2838  | 4.48                      | 0.12         | 8.74           |                |              |      |                        |              |      |
| qFM-Chr26-3 | 2016 | Bin2845 Bin2846  | 5.55                      | 0.13         | 10.69          |                |              |      |                        |              |      |

<sup>a</sup>QTL noted by '*' referred to common QTL detected on two datasets at least two years
<sup>b</sup>Phenotypic variation explained by a single locus QTL
<sup>c</sup>E1, salt stress condition; E2, normal condition; D-value, the difference values between salt stress and normal conditions
Pleiotropic effects analysis on FL and FS

A total of 35 clusters located on 18 chromosomes showed pleiotropic effects involving 107 QTL (Table 6). Of these, four clusters existed on chromosome (Chr) 5, Chr7, and Chr24; three were detected on Chr15 and Chr25; two were detected on Chr8, Chr14, Chr16, and Chr21, and only one cluster was detected on other 9 chromosomes.

There are two pleiotropic regions that can improve five fiber quality traits, which are Loci-Chr1-1 and Loci-Chr14-1, respectively. Two clusters could improve four fiber quality traits, which including Loci-Chr15-1 (FL, FU, FE, FM), Loci-Chr24-2 (FL, FU, FE, FS). Six clusters controlled three traits, which were Loci-Chr3-2 (FL, FS, FM), Loci-Chr7-4 (FU, FS, FE), Loci-Chr19-1 (FU, FS, FM), Loci-Chr21-2 (FL, FE, FM), Loci-Chr23-1 (FL, FE, FM), and Loci-Chr25-2 (FS, FE, FM), respectively.

Among the 35 pleiotropic regions, six affected FL and FS simultaneously, which were distributed on Chr5, Chr7, Chr14, and Chr24, of which two regions were found in both Chr5 and Chr24. Loci-Chr3-1, Loci-Chr5-4, and Loci-Chr7-1, controlled FL and FS simultaneously and received favorable alleles contributed by the female GX1135. Among them, the two QTL qFS-Chr5-1 and qFS-Chr5-2 that controlled FS on Loci-Chr5-1 were detected in E1 and E2 in 2016 and 2017. The two QTL qFL-Chr5-1 and qFL-Chr5-2 that control FL were detected in E1 and E2 in 2016 and 2017 simultaneously. The cluster also controlled FU, FE, and FM. The Loci-Chr5-4 cluster contains six QTL, of which three QTL controlled FS (qFS-Chr5-1, qFS-Chr5-6 and qFS-Chr5-7), one controlled FL (qFL-Chr5-5), which detected on Chr5 and Chr25; two regions were found in both Chr5 andChr24. Loci-Chr3-1, Loci-Chr5-4, and Loci-Chr7-1, controlled FL and FS simultaneously and received favorable alleles contributed by the female GX1135. Among them, the two QTL qFS-Chr5-1 and qFS-Chr5-2 that controlled FS on Loci-Chr5-1 were detected in E1 and E2 in 2016 and the two QTL qFL-Chr5-1 and qFL-Chr5-2 that control FL were detected in E1 and E2 in 2016 and 2017 simultaneously. The cluster also controlled FU, FE, and FM. The Loci-Chr5-4 cluster contains six QTL, of which three QTL controlled FS (qFS-Chr5-1, qFS-Chr5-6 and qFS-Chr5-7), one controlled FL (qFL-Chr5-5), which detected in E1 and E2 in 2016 and 2017, and the region also controls FM (qFM-Chr5-3). Two QTL were detected on Loci-Chr7-1, of which, qFS-Chr7-1, affecting FS was detected in E2 in 2017, but qFL-Chr7-1 was detected in E3 in 2016. Loci-Chr24-2 and Loci-Chr24-3, two pleiotropic regions on chromosome 24, were detected in both E1 and E2 in 2016 and 2017, and the favorable alleles were contributed by male parent GX100-2. Among them, two QTL influencing FL and FS in Loci-Chr24-2, (qFS-Chr24-1, qFS-Chr24-2 and qFL-Chr24-2, qFL-Chr24-3), which also control FE and FU simultaneously. Eight QTL were detected in Loci-Chr14-1, of which only one QTL (qFS-Chr14-1) influencing FS was detected in E2, 2016, two QTL from Loci-Chr14-1 influencing FL were detected in E2, 2018. The favorable alleles in qFS-Chr14-1 and qFL-Chr14-2 originated from the female GX1135, while the favorable alleles in qFL-Chr14-3 were contributed by male GX100-2.

Identification of candidate genes within QTL and expression validation by RNA-seq

In this study, two QTL (qFL-Chr1-1 and qFL-Chr5-5) influencing FL were detected in both E1 and E2 and detected repeatedly across two years. Explaining 4.26% of the average PV, qFL-Chr1-1, is favorably provided by the female parent. The QTL, qFL-Chr5-5, is provided by male parent, and the average PV is 9.38%. Among them, the confidence interval of qFL-Chr1-1 is between marker Bin28 and Bin29, corresponding to the reference genome of upland cotton TM-1 10,876,918 bp to 11,016,009 bp, the physical distance of this interval is 139,091 bp, which includes 12 annotated genes (Gh_A01G0604-Gh_A01G0615). The flanking markers of qFL-Chr5-5 are Bin648 and Bin649, corresponding to the reference genome of upland cotton 88,623,793 bp to 88,671,394 bp, the physical distance of this region is 47,601 bp, including two annotated genes (Gh_A05G3395 and Gh_A05G3396) (Table 7). Among them, there are 11 known genes and three genes were with uncharacterized protein information. The known genes were annotated as Microtubule-associated protein TORTIFOLIA1, DNA polymerase I A, UDP-glycosyltransferase 73C5, Serine/threonine-protein kinase PBS1, Vacular protein sorting-associated protein, Fatty acid desaturase 4, Cytochrome P450 716B1, Protein FATTY ACID EXPORT 5, Polyadenylate-binding protein RBP45C, Probable protein phosphatase 2C 33, and Aspartic proteinase-like protein 2, respectively. These putative candidate genes were explored for the public RNA-seq expression data of TM-1 in fiber development (Fig. 1). The result showed that fragments per kilobase million (FPKM) of four genes (Gh_A01G0606, Gh_A01G0607, Gh_A01G0611, and Gh_A01G0613) were less than 1. The gene (Gh_A01G0615) is persistently highly expressed at different stages of fiber development. Three genes (Gh_A01G0604, Gh_A01G0605, and Gh_A01G3396) were highly expressed at various stages of fiber development (5, 10, and 20 DPA).

To validate the potential function in fiber development, the expression pattern of candidate genes was verified with qRT-PCR using fibers of extremely long fiber line (H), extremely short fiber line (L), female (GX1135, F), male (GX100-2, M) at different developmental stages (Fig. 2). The expression of Gh_A01G0604 in extremely long fiber line was six times higher than that in extremely short fiber line at 5 DPA. The expression of Gh_A01G0610, Gh_A01G0612, Gh_A01G0615, Gh_A01G3395, and Gh_A01G3396 in extremely long fiber line was significantly higher than that of extremely short fiber line at many points from 10 to 20 DPA. Therefore, these seven genes were identified as candidate genes responsible for influencing fiber cell elongation.
Table 6 Pleiotropic regions in the present study for fiber quality traits

| Cluster       | QTL          | Year | Flanking markers | Under E1<sup>c</sup> | Under E2 | Mapping by D-value |
|---------------|--------------|------|------------------|-----------------------|----------|---------------------|
|               |              |      |                  | LOD | Additive Var% | LOD | Additive Var% | LOD | Additive Var% |
| Loci-Chr2-1   | qFE-Chr2-1   | 2017 | Bin128 Bin129    | 2.95 | − 0.02 | 6.44 |
|               | qFM-Chr2-1   | 2017 | Bin128 Bin129    | 2.71 | − 0.06 | 4.02 |
|               | qFS-Chr2-1   | 2017 | Bin139 Bin140    | 2.26 | − 0.32 | 4.11 |
| Loci-Chr4-1   | qFU-Chr4-1   | 2017 | Bin472 Bin473    | 3.10 | − 0.26 | 6.48 |
|               | qFM-Chr4-2   | 2017 | Bin484 Bin485    | 3.99 | − 0.07 | 5.72 |
| Loci-Chr5-1   | qFM-Chr5-1   | 2017 | Bin488 Bin489    | 2.21 | 0.05  | 2.91 |
|               | qFU-Chr5-1   | 2017 | Bin492 Bin493    | 2.66 | − 0.20 | 5.18 |
|               | qFE-Chr5-1   | 2016 | Bin493 Bin494    | 5.49 | − 0.03 | 11.13 |
|               | qFS-Chr5-1   | 2016 | Bin488 Bin489    | 3.60 | − 0.31 | 6.50 |
|               | qFS-Chr5-2   | 2016 | Bin500 Bin501    | 2.31 | − 0.25 | 4.24 |
|               | qFL-Chr5-2   | 2016 | Bin500 Bin501    | 2.31 | − 0.25 | 4.04 |
|               | qFL-Chr5-1   | 2016 | Bin490 Bin491    | 4.61 | − 0.34 | 8.20 |
| Loci-Chr5-2   | qFL-Chr5-3   | 2016 | Bin512 Bin513    | 5.87 | − 0.37 | 10.31 |
|               | qFL-Chr5-4   | 2017 | Bin515 Bin516    | 6.23 | − 0.36 | 11.06 |
|               | qFL-Chr5-5<sup>a</sup> | 2016 | Bin527 Bin528    | 6.90 | − 0.38 | 12.14 |
|               | qFE-Chr5-2   | 2016 | Bin521 Bin522    | 3.26 | 0.02  | 6.42 |
|               | qFE-Chr5-3   | 2016 | Bin530 Bin531    | 2.13 | 0.02  | 4.36 |
| Loci-Chr5-3   | qFS-Chr5-3   | 2017 | Bin548 Bin549    | 2.22 | − 0.33 | 3.90 |
|               | qFU-Chr5-2   | 2016 | Bin554 Bin555    | 2.45 | 0.22  | 4.99 |
| Loci-Chr5-4   | qFS-Chr5-5<sup>a</sup> | 2016 | Bin638 Bin639    | 2.98 | − 0.29 | 5.61 |
|               | qFS-Chr5-6   | 2017 | Bin640 Bin641    | 3.98 | − 0.45 | 7.06 |
|               | qFS-Chr5-7   | 2017 | Bin653 Bin654    | 5.75 | − 0.54 | 9.98 |
|               | qFS-Chr5-8<sup>a</sup> | 2016 | Bin655 Bin656    | 5.75 | − 0.40 | 10.42 |
|               | qFL-Chr5-5<sup>a</sup> | 2016 | Bin648 Bin649    | 5.91 | − 0.39 | 10.80 |
|               | qFM-Chr5-2<sup>*</sup> | 2017 | Bin648 Bin649    | 3.29 | − 0.26 | 5.68 |
| Loci-Chr7-1   | qFS-Chr7-1   | 2017 | Bin789 Bin790    | 2.36 | − 0.31 | 4.35 |
|               | qFL-Chr7-1   | 2016 | Bin795 Bin796    | 2.49 | 0.23  | 5.57 |
| Loci-Chr7-2   | qFM-Chr7-1   | 2016 | Bin842 Bin843    | 2.53 | 0.08  | 4.51 |
|               | qFU-Chr7-2   | 2016 | Bin850 Bin851    | 2.16 | 0.08  | 3.95 |
| Loci-Chr7-3   | qFU-Chr7-3   | 2018 | Bin867 Bin868    | 2.22 | − 0.20 | 4.59 |
|               | qFS-Chr7-3   | 2018 | Bin868 Bin869    | 2.27 | − 0.41 | 5.11 |
| Loci-Chr7-4   | qFE-Chr7-1   | 2018 | Bin872 Bin873    | 3.33 | − 0.01 | 6.80 |
|               | qFS-Chr7-4   | 2017 | Bin880 Bin881    | 2.10 | − 0.33 | 3.74 |
|               | qFU-Chr7-4   | 2018 | Bin881 Bin882    | 2.05 | − 0.19 | 4.25 |
| Loci-Chr8-1   | qFM-Chr8-1   | 2018 | Bin910 Bin911    | 2.84 | 0.08  | 5.85 |
|               | qFS-Chr8-1   | 2018 | Bin912 Bin913    | 2.27 | − 0.37 | 4.85 |
Table 6 (continued)

| Cluster  | QTL     | Year | Flanking markers | Under E1<sup>c</sup> | Under E2 | Mapping by D-value |
|----------|---------|------|------------------|-----------------------|----------|--------------------|
|          |         |      |                  | LOD | Additive | Var%<sup>b</sup> | LOD | Additive | Var% |
| Loci-Chr8-2 | qFM-Chr8-3* | 2017 | Bin964 Bin965 | 2.73 | 0.06 | 4.04 | 2016 | Bin969 Bin970 | 3.48 | 0.10 | 6.71 |
|          |         | 2016 | Bin970 Bin971 | 2.94 | 0.09 | 5.48 | 2018 | Bin977 Bin978 | 2.34 | -0.07 | 4.85 |
|          |         | 2018 | Bin977 Bin978 | 3.46 | -0.09 | 7.30 | 2017 | Bin977 Bin978 | 2.02 | 0.06 | 3.01 |
|          |         | 2018 | Bin977 Bin978 | 3.46 | -0.09 | 7.30 | 2018 | Bin982 Bin983 | 2.84 | -0.07 | 5.86 |
| qFS-Chr8-2 |         | 2017 | Bin975 Bin976 | 3.44 | -0.42 | 6.14 | 2017 | Bin980 Bin981 | 2.75 | -0.38 | 5.00 |
| Loci-Chr12-1 | qFE-Chr12-1 | 2016 | Bin1364 Bin1365 | 2.08 | 0.02 | 4.65 |
|          | qFL-Chr12-1 | 2018 | Bin1368 Bin1369 | 2.97 | -0.22 | 6.17 |
|          |         | 2018 | Bin1371 Bin1372 | 2.72 | 0.27 | 5.45 |
| Loci-Chr13-1 | qFU-Chr13-2 | 2017 | Bin1449 Bin1450 | 2.55 | 0.20 | 5.03 |
|          | qFU-Chr13-3 | 2018 | Bin1467 Bin1468 | 2.15 | 0.30 | 4.54 |
|          | qFM-Chr13-2 | 2018 | Bin1456 Bin1457 | 2.03 | 0.04 | 4.44 |
|          | qFM-Chr13-3 | 2016 | Bin1473 Bin1474 | 2.20 | 0.09 | 3.84 |
| Loci-Chr14-1 | qFE-Chr14-1 | 2018 | Bin1494 Bin1495 | 3.65 | -0.01 | 7.66 |
|          | qFE-Chr14-2 | 2018 | Bin1508 Bin1509 | 4.60 | -0.01 | 9.54 |
|          |         | 2018 | Bin1516 Bin1517 | 3.17 | -0.01 | 6.68 |
|          | qFS-Chr14-1 | 2016 | Bin1501 Bin1502 | 3.08 | 0.30 | 5.42 |
|          | qFU-Chr14-1 | 2018 | Bin1520 Bin1521 | 3.52 | 0.26 | 8.16 |
|          | qFM-Chr14-1 | 2017 | Bin1502 Bin1503 | 3.11 | -0.07 | 4.59 |
|          | qFM-Chr14-2 | 2016 | Bin1521 Bin1522 | 2.54 | -0.10 | 5.57 |
|          |         | 2016 | Bin1528 Bin1529 | 2.37 | -0.09 | 4.99 |
|          | qFL-Chr14-2 | 2018 | Bin1517 Bin1518 | 2.13 | -0.23 | 4.01 |
|          | qFL-Chr14-3 | 2016 | Bin1529 Bin1530 | 2.13 | 0.21 | 4.40 |
| Loci-Chr14-2 | qFM-Chr14-4 | 2016 | Bin1550 Bin1551 | 4.63 | -0.12 | 8.82 |
|          | qFE-Chr14-3 | 2018 | Bin1553 Bin1554 | 2.25 | 0.01 | 4.60 |
| Loci-Chr15-1 | qFM-Chr15-1* | 2016 | Bin1568 Bin1569 | 2.08 | -0.08 | 4.11 |
|          |         | 2018 | Bin1571 Bin1572 | 3.55 | -0.06 | 7.37 |
|          |         | 2016 | Bin1579 Bin1580 | 3.84 | -0.11 | 7.42 |
|          |         | 2016 | Bin1580 Bin1581 | 3.38 | 0.11 | 7.22 |
|          |         | 2016 | Bin1589 Bin1590 | 2.48 | -0.09 | 4.88 |
|          |         | 2018 | Bin1594 Bin1595 | 4.94 | 0.07 | 10.49 |
|          | qFL-Chr15-1 | 2017 | Bin1599 Bin1600 | 2.47 | -0.23 | 5.15 |
|          | qFU-Chr15-1 | 2016 | Bin1579 Bin1580 | 2.97 | -0.18 | 5.70 |
|          | qFU-Chr15-2 | 2016 | Bin1599 Bin1600 | 2.65 | 0.23 | 5.54 |
|          | qFE-Chr15-1 | 2016 | Bin1589 Bin1590 | 2.04 | 0.02 | 4.52 |
|          |         | 2016 | Bin1596 Bin1597 | 2.80 | 0.02 | 5.68 |
|          |         | 2016 | Bin1599 Bin1600 | 2.08 | 0.02 | 4.22 |
| Loci-Chr15-2 | qFS-Chr15-1 | 2017 | Bin1659 Bin1660 | 2.14 | 0.29 | 3.73 |
|          | qFM-Chr15-2 | 2017 | Bin1678 Bin1679 | 2.02 | -0.05 | 2.84 |
Table 6 (continued)

| Cluster       | QTL          | Year | Flanking markers | Under E1<sup>c</sup> | Under E2 | Mapping by D-value |
|---------------|--------------|------|------------------|----------------------|----------|-------------------|
|               |              |      |                  | LOD | Additive | Var%<sup>b</sup> | LOD | Additive | Var% |
| Loci-Chr15-3 | qFM-Chr15-3  | 2017 | Bin1689 Bin1690  | 5.53 | −0.10    | 9.13         |     |          |      |
|               |              | 2016 | Bin1690 Bin1691  | 3.48 | −0.10    | 6.72         |     |          |      |
|               |              | 2017 | Bin1690 Bin1691  | 2.48 | −0.06    | 3.84         |     |          |      |
| Loci-Chr16-1 | qFE-Chr16-1  | 2017 | Bin1670 Bin1708  | 2.02 | 0.23     | 4.00         |     |          |      |
|               | qFL-Chr16-1  | 2016 | Bin1707 Bin1708  | 2.02 | 0.23     | 4.00         |     |          |      |
| Loci-Chr16-2 | qFL-Chr16-2  | 2017 | Bin1784 Bin1785  | 2.10 | −0.20    | 3.38         |     |          |      |
|               | qFM-Chr16-1<sup>*</sup> | 2016 | Bin1817 Bin1818  | 2.03 | −0.09    | 4.38         | 2015 | Bin1817 Bin1818 | 2.03 | −0.09    | 4.38 |
| Loci-Chr18-1 | qFU-Chr18-1  | 2016 | Bin1904 Bin1905  | 2.45 | 0.20     | 5.13         |     |          |      |
|               | qFS-Chr18-1  | 2017 | Bin1909 Bin1910  | 3.33 | 0.39     | 6.51         |     |          |      |
| Loci-Chr19-1 | qFU-Chr19-1  | 2017 | Bin2057 Bin2058  | 2.28 | −0.17    | 4.97         |     |          |      |
|               | qFM-Chr19-1  | 2017 | Bin2061 Bin2062  | 3.00 | −0.05    | 6.15         |     |          |      |
|               | qFS-Chr19-1  | 2017 | Bin2066 Bin2067  | 2.75 | 0.36     | 5.35         |     |          |      |
| Loci-Chr21-1 | qFM-Chr21-1  | 2017 | Bin2279 Bin2280  | 2.05 | −0.05    | 2.71         |     |          |      |
|               |              | 2016 | Bin2285 Bin2286  | 2.00 | 0.08     | 3.77         |     |          |      |
|               |              | 2016 | Bin2292 Bin2293  | 2.77 | 0.09     | 5.15         |     |          |      |
| Loci-Chr21-2 | qFM-Chr21-2  | 2017 | Bin2330 Bin2331  | 2.75 | 0.06     | 5.77         |     |          |      |
|               | qFE-Chr21-2  | 2017 | Bin2336 Bin2337  | 2.56 | 0.02     | 4.76         |     |          |      |
|               | qFE-Chr21-3  | 2017 | Bin2345 Bin2346  | 2.28 | 0.02     | 4.37         |     |          |      |
|               | qFL-Chr21-1  | 2017 | Bin2335 Bin2336  | 2.84 | 0.24     | 4.87         |     |          |      |
|               | qFL-Chr21-2  | 2016 | Bin2338 Bin2339  | 3.19 | 0.28     | 5.65         |     |          |      |
|               |              | 2016 | Bin2347 Bin2348  | 2.25 | 0.24     | 4.10         |     |          |      |
| Loci-Chr22-1 | qFS-Chr22-1  | 2018 | Bin2411 Bin2412  | 2.72 | 0.52     | 6.64         |     |          |      |
|               | qFE-Chr22-2  | 2017 | Bin2412 Bin2413  | 2.55 | 0.02     | 4.44         |     |          |      |
| Loci-Chr23-1 | qFL-Chr23-1  | 2018 | Bin2500 Bin2501  | 3.22 | 0.22     | 6.72         |     |          |      |
|               | qFL-Chr23-2  | 2018 | Bin2503 Bin2504  | 2.11 | 0.24     | 4.73         |     |          |      |
|               | qFM-Chr23-1  | 2017 | Bin2537 Bin2538  | 2.06 | 0.05     | 2.73         |     |          |      |
|               | qFU-Chr23-1  | 2017 | Bin2554 Bin2555  | 2.05 | 0.21     | 4.38         |     |          |      |
| Loci-Chr24-1 | qFU-Chr24-1  | 2016 | Bin2558 Bin2559  | 3.37 | −0.30    | 6.93         |     |          |      |
|               | qFL-Chr24-1  | 2017 | Bin2558 Bin2559  | 2.67 | 0.26     | 4.74         |     |          |      |
| Loci-Chr24-2 | qFS-Chr24-1  | 2017 | Bin2578 Bin2579  | 6.25 | 0.56     | 11.19        |     |          |      |
|               |              | 2016 | Bin2584 Bin2585  | 4.06 | 0.35     | 7.94         |     |          |      |
|               |              | 2017 | Bin2579 Bin2580  | 2.90 | 0.35     | 5.39         |     |          |      |
|               | qFS-Chr24-2  | 2017 | Bin2592 Bin2593  | 7.19 | 0.60     | 12.73        |     |          |      |
|               | qFE-Chr24-1  | 2018 | Bin2584 Bin2585  | 3.70 | 0.01     | 7.59         |     |          |      |
|               | qFU-Chr24-2  | 2016 | Bin2586 Bin2587  | 2.23 | 0.24     | 4.52         |     |          |      |
|               | qFL-Chr24-2  | 2016 | Bin2581 Bin2582  | 2.66 | 0.25     | 4.52         |     |          |      |
|               |              | 2017 | Bin2587 Bin2588  | 2.61 | 0.26     | 4.67         |     |          |      |
|               | qFL-Chr24-3  | 2016 | Bin2593 Bin2594  | 4.08 | 0.31     | 6.80         |     |          |      |
Genes within QTL for FL conferring fatty acid synthesis and degradation

Among the 40 QTL identified for FL, 9 were detectable in D-value, 13 positive and 18 negative effect QTL among the remaining 31 QTL were observed on E1 or E2, respectively. This examination failed to find the corresponding physical location of some SSR markers on the genome, 10 of which were QTL with positive effect and 11 QTL with negative effect for FL were subjected to gene functional prediction. The physical locations of these QTL on the reference genome of *G. hirsutum* were found, and the numbers of genes in the corresponding regions were 977 and 791, respectively (Table S7).

We annotated these genes by GO and classified the annotated GO terms. Among them, genes in the QTL region of positive and negative effects were divided into 52 subgroups, belonging to three major categories, CC (cellular components), MF (molecular function), and BP (biological processes) (Fig. S2). There was no significant difference between genes with positive and negative effect QTL in GO classification. GO enrichment of these two kinds of genes showed that the positive effect genes were mainly enriched in three GO items: transmembrane receptor protein tyrosine kinase activity in BP, cell periphery in CC, and system development in MF. The negative effect genes were mainly enriched in abscisic acid glucosyltransferase of BP, membrane-bounded organelle in CC and cellular protein modification processes in MF (Fig. S3).

To better understand the biological function of candidate genes and their metabolic pathways, all genes (70,478) in upland cotton (Zhang et al. 2015) serve as background and the candidate genes in positive and negative effect QTL were annotated and enriched by KEGG (Fig. 3 and Fig. S4). KEGG annotation showed that the genes in positive QTL were mainly concentrated in three metabolic pathways: plant hormone signal transduction, carbon metabolism and ribosomal, while the genes in negative effect QTL were mainly enriched in plant hormone signal transduction, carbon metabolism, plant pathogen interaction, ribosomes and endocytosis. Interestingly, we found that genes with positive and negative effect QTL are involved in fatty acid metabolism pathways, in which positive effect genes participate in ko00061 (Fatty acid biosynthesis), ko00062 (Fatty acid elongation) and ko01040 (Biosynthesis of unsaturated fatty acids); negative effect genes participate in ko00071 (Fatty acid degradation) (Fig. 4). There are two genes (*Gh_D08G1373* and *Gh_D08G1533*) involved in fatty acid elongation pathway six genes involved in fatty acid biosynthesis pathway and two genes involved in fatty acid degradation pathway (Table S8).

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**Table 6 (continued)**

| Cluster  | QTL          | Year | Flanking markers | Under E1 | Under E2 | Mapping by D-value |
|----------|--------------|------|-----------------|----------|----------|--------------------|
|          |              |      |                 | LOD      | Additive Var% | LOD      | Additive Var% | LOD      | Additive Var% |
| Loci-Chr24-3 | *qFS-Chr24-3* | 2016 | Bin2601 Bin2602 | 4.78     | 0.51      | 8.72    |
|          | *qFL-Chr24-4* | 2017 | Bin2604 Bin2605 | 2.27     | 0.25      | 4.09    | 2.13     | 0.23      | 3.64    |
|          |              |      |                 | 2.10     | 0.22      | 4.47    |
| Loci-Chr24-4 | *qFS-Chr24-5* | 2017 | Bin2627 Bin2628 | 2.74     | 0.19      | 5.78    |
|          | *qFS-Chr24-3* | 2017 | Bin2630 Bin2631 | 2.07     | 0.21      | 4.35    |
| Loci-Chr25-1 | *qFS-Chr25-1* | 2017 | Bin2674 Bin2675 | 2.20     | 0.18      | 4.54    |
|          | *qFS-Chr25-1* | 2016 | Bin2681 Bin2682 | 2.06     | 0.08      | 4.32    |
|          |              |      |                 | 2.90     | 0.02      | 5.95    |
| Loci-Chr25-2 | *qFS-Chr25-1* | 2016 | Bin2700 Bin2701 | 2.48     | 0.09      | 4.91    |
|          |              |      |                 | 2.06     | 0.08      | 4.56    |
| Loci-Chr25-3 | *qFS-Chr25-2* | 2016 | Bin2707 Bin2708 | 2.03     | 0.24      | 3.66    |
|          |              |      |                 | 2.67     | 0.28      | 4.78    |
| Loci-Chr25-5 | *qFS-Chr25-1* | 2018 | Bin2763 Bin2764 | 2.70     | 0.31      | 5.72    |
|          |              |      |                 | 2.76     | 0.32      | 5.85    |
| Loci-Chr26-1 | *qFS-Chr26-1* | 2016 | Bin2808 Bin2809 | 2.09     | 0.23      | 3.65    |
|          |              |      |                 | 2.37     | 0.08      | 4.52    |

*a QTL noted by ‘*’ referred to common QTL detected on two datasets at least two years

*b Phenotypic variation explained by a single locus QTL

*c E1, salt stress condition; E2, normal condition; D-value, the difference values between salt stress and normal conditions
Table 7 Putative candidate genes identified within important QTL genomic regions

| QTL        | Gene ID   | Gene name   | Description                                      |
|------------|-----------|-------------|--------------------------------------------------|
| qFL-Chr01-1| Gh_A01G0604 | TOR1        | Microtubule-associated protein TORTIFOLIA1       |
|            | Gh_A01G0605 | POLIA       | DNA polymerase I A, chloroplastic/mitochondrial  |
|            | Gh_A01G0606 | UGT73C5     | UDP-glycosyltransferase 73C5                     |
|            | Gh_A01G0607 | PBS1        | Serine/threonine-protein kinase PBS1             |
|            | Gh_A01G0608 | NA          | NA                                                |
|            | Gh_A01G0609 | NA          | NA                                                |
|            | Gh_A01G0610 | VP22-1      | Vacuolar protein sorting-associated protein 22 homolog 1 |
|            | Gh_A01G0611 | FAD4        | Fatty acid desaturase 4, chloroplastic           |
|            | Gh_A01G0612 | NA          | NA                                                |
|            | Gh_A01G0613 | CYP716B1    | Cytochrome P450 716B1                            |
|            | Gh_A01G0614 | FAX5        | Protein FATTY ACID EXPORT 5                      |
|            | Gh_A01G0615 | RBP45C      | Polyadenylate-binding protein RBP45C             |
| qFL-Chr05-5| Gh_A05G3395 | PPC6-1      | Probable protein phosphatase 2C 33              |
|            | Gh_A05G3396 | At1g65240   | Aspartic proteinase-like protein 2               |

Discussion

The salinity effects on cotton growth and fiber quality traits

According to the classification standard of soil salt content, in the field trials from 2016 to 2018, the classification of soil salinity is moderate salinization in salt stress condition, and mild salinization in normal condition, as well as the depth of 0–20 cm (Table S1, S2). It can be preliminarily considered that salt treatment is consistent and effective.

By observing the cotton performance in the field, we found that compared with the cotton planted in control soil, the plant height of cotton grown in saline soil was shorter, and leaf margins curled and wilted along the leaf vein. We saw that salt stress negatively affected the vegetative growth of cotton (Fig. S2). The tendency of fiber quality to change among lines in the RIL population under E1 and E2 was different from 2016 to 2018. This could be due to the increase in soil salt content in 2018 and the uneven distribution of salt in the experimental area. It has been reported that low salinity can promote plant growth, but little research has been published to expound the molecular mechanisms of the effects of salt stress on fiber quality in cotton. Salt stress has been shown to result in a considerable decrease in the fresh and dry weights of roots, stems, and leaves (Chartzoulakis and Klapaki 2000; Su et al. 2020). It is reported that in *Alhagi pseudoalhagi* (a leguminous plant), plant weight increased at low salinity (50 mM NaCl) but decreased at high salinity (100 and 200 mM NaCl) (Kurban H. et al. 1999).

QTL conferring fiber quality under salt stress

The identification of QTL across multiple environments and populations plays an essential role in marker-assisted selection (MAS) (Jamshed et al. 2016). A total of 318 QTL for FL in upland cotton have been submitted in CottonQTLdb database (https://www.cottonqtlodb.org:8081). In the present study, 12 QTL were detected over two years, of which seven, one, four, and one QTL were detected for FL, FU, FS, FE, and FM. The QTL detected in at least two datasets during the same year were defined as stable QTL. All of 10 stable QTL showed effect values by alleles with different parents. There are two stable QTL underlying FL (qFL-Chr1-1 and qFL-Chr24-4), one (qFS-Chr25-2) controlling...
FS and three (qFM-Chr5-2, qFM-Chr8-2 and qFM-Chr25-1) for FM were provided positive provided alleles by the female parent. A total of six QTL were detected in both E1 and E2. Three QTL controlling FL were detected in 2016, of which, qFL-Chr1-1 and qFL-Chr24-4 were provided alleles by the female parent and qFL-Chr5-5 was provided alleles positive by the male parent. Two of the QTL that affect FM are located on Chr8 (qFM-Chr8-2, qFM-Chr8-3) and were detected in 2018. One stable QTL (qFM-Chr5-2) was detected in 2017, where qFM-Chr5-2 and qFM-Chr8-2 demonstrated positive additive effects, and the other QTL, qFM-Chr8-3 showed negative additive effects. The results revealed that these hotspots play important roles in two conditions for diverse fiber quality traits but different mechanisms responded between under E1 and E2.

Additionally, two QTL (qFM-Chr25-1, qFS-Chr25-2) were detected in E1 and D-value in 2016. The favorable alleles were provided by the female parent for both FM and FS. Two QTL were detected in E2 and D-value in 2016, affecting FM, qFM-Chr15-1, the positive allele of which was provided by the male parent and qFM-Chr16-1, the positive allele of which was provided by the female parent. A total of 46 QTL were detected for abiotic and biotic stress resistance in cotton on Chr5 including three hotspots, and 22 QTL on Chr14 including 1 hotspot, and 31 QTL on Chr15 including 2 hotspots (Abdelraheem et al. 2017). The hotspot named
c15-ST-Hotspot-1 was detected for fresh root weight under salt stress condition. The stable QTL, qFE-Chr25-1 in the present study, was detected in D-value, consistently with the same chromosome harboring a hotspot for Micronaire under normal irrigation condition (Said et al. 2013) and two hotspots for abiotic and biotic stress resistance underlying Micronaire and fiber elongation in cotton (Abdelraheem et al. 2017). This indicates regions on the same chromosome underlying fiber quality traits showed response to diverse stress conditions. One consistent QTL located on Chr9 and four consistent QTL on Chr15 were detected for shoot height, shoot fresh weight, shoot dry weight, and root dry weight under salt stress in seedling stage (Oluoch et al. 2016).

**Stable QTL affecting FL under multiple environments across multiple years**

A QTL cluster is defined as a densely populated QTL region on a chromosome that contains many QTL associated with different traits (Rong et al. 2007). In this study, we identified 35 clusters located on 18 chromosomes. Two stable QTL were located in the cluster, Loci-Chr5-4 controlling FL (qFL-Chr5-5) and FM (qFM-Chr5-2) under E1 and E2. This cluster harbored six QTL, of which three QTL controlled FS (qFS-Chr5-5, qFS-Chr5-6, qFS-Chr5-7) and one for FL (qFL-Chr5-5) had negative additive effects, while two QTL qFM-Chr5-2 and qFM-Chr5-3 controlled FM showed positive additive effects. Three pleiotropic regions contained two stable QTL (qFM-Chr25-1 and qFS-Chr25-2) were detected on Chr25, of which it also controlled FL and FU simultaneously. This is consistent with previous research that an important cluster with more than three traits, with high broad sense heritability and high percentage of phenotypic variation was identified on Chr25 (Diouf et al. 2018). There were four QTL-clusters on Chr24, six QTL in Loci-Chr24-2, and two QTL in Loci-Chr24-1, Loci-Chr24-3, Loci-Chr24-4, respectively. In Loci-Chr24-2, six QTL had positive additive effects which controlled FL, FS, FE, and FU simultaneously. One stable QTL, qFL-Chr24-4, had positive additive effects that were identified in Loci-Chr24-3 on both E1 and E2, which controlled FS.

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**Fig. 4** Genes involved in fatty acid metabolism. a Genes involved in fatty acid elongation pathway. b Genes involved in fatty acid biosynthetic pathway. c Genes involved in fatty acid degradation pathway.
(2019) found 12 genes possessing non-synonymous SNPs (nsSNPs) significantly associated with fiber length on Chr. D11 (Chr24 in present research) using 550 RILs derived from eleven different cultivars. The high correlation of the QTL detected in this study to the previous finding, provides the opportunity for the utilization of these QTL in MAS to improve the fiber quality of upland cotton.

There were six QTL-clusters influencing FL and FS, of which two clusters (Loci-Chr5-1, Loci-Chr5-4) located on Chr5, two (Loci-Chr24-2, Loci-Chr24-1) located on Chr24, one (Loci-Chr7-1) located on Chr7, and one (Loci-Chr14-1) located on Chr14, respectively. Six clusters affecting FL and FS simultaneously received favorable alleles contributed by the same parent under E1 and E2 except Loci-Chr14-1 (Table 6). The cluster located on Chr14 indicated that the correlation between FS and FL was not altered by salt stress.

**Advantage of multiple datasets for QTL mapping under salt stress**

In general, when mapping with composite traits, the QTL detected by both constituent and composite traits have a positive correlation in detecting efficacy. Constituent traits can accumulate the genetic effect; however, the efficiency of composite traits is generally high with multiplier effects (Hua et al. 2002). In recent years, composite traits in stress response have been studied using QTL mapping strategies. In a maize example, anthesis silking interval (ASI) index was used to identify the phenotypic value for drought tolerance (Ribaut et al. 1996; Messmer et al. 2009). ASI in tropical open-pollinated varieties was negatively correlated with yields under drought stress, so ASI was one of the most important composite traits in the identified drought tolerance. The phenotypic values of ASI came from the differences between MFLW (male flowering) and FFLW (female flowering). Ribaut et al. (1996) used 142 molecular markers to analyze the characters of MFLW, FFLW, and ASI of a maize F2 population under good irrigation and water stress conditions, respectively. In the present study, the D-value was used to represent the effect of salt stress on the RIL population, which was developed as a compound trait. The results showed that 46 QTL were detected in D-value, of which 11 could be detected in the constituent traits explaining from 4.32 to 11.19% of PV and LOD values ranged 2.06–5.28. Of the 11 QTL, four QTL (qFS-Chr25-2, qFM-Chr15-1, qFM-Chr16-1, qFM-Chr25-1) were detected using constituent trait and composite trait in the same year (E1 and D-value, E2 and D-value), which explained PV, ranging from 4.32% to 6.59% (Table 5). Among them, two QTL (qFM-Chr15-1, qFM-Chr16-1) were detected on E2 and D-value, and two (qFM-Chr25-1, qFS-Chr25-2) were detected on E1 and D-value. This research exploits and provides an example for QTL effects detected in both the composite trait and the constituent traits for salt-tolerant QTL mapping.

**Candidate genes positively regulate fiber elongation**

Fiber length is one of the most important and highly inheritable fiber quality trait, which has been successfully used for genetic analysis in cotton and is directly related to its spinning quality. Fiber development is a complex physiological and biochemical process, which goes through multiple stages and involves a multi-level and multi-pathway molecular regulation network. Among them, plant hormones, turgor regulation, and cytoskeleton can all participate in the regulation of cotton fiber growth and development (Ascencio-Ibáñez et al. 2008; Gardiner et al. 2011). Two genes, Gh_A05G3395 and Gh_A05G3396, located within qFL-Chr5-5 had homologous genes in *Arabidopsis thaliana*. Gh_A05G3396 was a membrane-anchored aspartic protease, which contributed to pollen and ovule development. And Gh_A05G3395 is an Mn$^{2+}$ or Mg$^{2+}$-dependent protein serine/threonine phosphatase, which may constitute positive regulators in ABA-mediated signaling pathways (Vanholme et al. 2014; Xue et al. 2008). Li et al. (2002) cloned a gene (GhTUB1), which encoded β subunit of micro-tubulin and predominantly expressed in cotton fiber. He et al. (2008) identified 795 tubulin ESTs (expressed sequence tags), in upland cotton, out of which 19 β-TUB genes were cloned. The microtubule-associated protein encoding gene (Gh_A01G0604) was significant highly expressed in extremely long fiber length line at the earliest time of fiber development (5 DPA). Gh_A01G0605 (DNA polymerase I A), Gh_A01G0610 (vacuolar protein sorting-associated protein), Gh_A01G0612 (no functional annotation), Gh_A01G0615 (Polyadenylate-binding protein) in extremely long fiber line was significantly higher than that of extremely short fiber line at 10 DPA to 20 DPA. These candidate genes annotated as being associated with plant turgor regulation, cytoskeleton processes, DNA replication, and protein synthesis might positively regulate fiber elongation in A. thaliana or in G. hirsutum, and these two QTL may contribute to fiber quality breeding.

**Fiber cell elongation aroused fatty acid synthesis pathway under salt stress**

In total, 977 genes in 10 positive effect QTL and 791 genes in 10 negative effect QTL were used to analyze gene function and the biological processes (Table S7). Interestingly, we found that genes with positive effects participate in ko00061 (Fatty acid biosynthesis), ko00062 (Fatty acid elongation), and ko01040 (Biosynthesis of unsaturated fatty acids); while negative effect genes involved in ko00071
(Fatty acid degradation). Of these, a gene encoding 3-ketoacyl-CoA synthase 1 belonging to GhKCS1 (Gh_D08G1373) was involved in fatty acid elongation pathway (Fig. 3 and 4, Table S8). The biosynthesis of VLCFAs and their transport are required for fiber development (Hu et al. 2007). The heterologous gene KCS of cotton expressed in Arabidopsis thaliana had promoted the elongation of stem cells (Qin et al. 2007; Shi et al. 2006). Furthermore, increased biosynthesis of fatty acid during the fiber cell elongation period implicated that fatty acid was serve as precursors of cutin and cuticular wax in the process (Hu et al. 2019a, b; Fig. 4). We predict that QTL with positive effects on fiber length may increase fiber length by promoting the synthesis and elongation of fatty acids, while QTL with negative effects may be responsible for fatty acid degradation and thus inhibit elongation of fiber cells. Our research provides a foundation for future examination of the role of fatty acid in cotton fiber development under salt stress. However, the regulative mechanism of these genes in fiber elongation still needs to be further verified.

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Author contribution AHG performed two years’ experiments, all data analysis and prepared the manuscript. YS performed one-year experiment and data collection. BI and HSN attended bench work and discussion. YH attended discussion and revised the manuscript. YMW maintained experimental platform and performed bench work. JPH conceived the experiments, provided experimental platform and revised the manuscript. All authors approved of the final manuscript.

Data availability All data generated or analyzed in this study included in published article and additional files. All of our raw data are available as Supporting Information: Supplementary tables S3–S5.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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