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ORIGINAL PAPER

Molecular markers associated with the immature fiber (*im*) gene affecting the degree of fiber cell wall thickening in cotton (*Gossypium hirsutum* L.)

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Abstract Cotton fiber fineness and maturity measured indirectly as micronaire (MIC) are important properties of determining fiber grades in the textile market. To understand the genetic control and molecular mechanisms of fiber fineness and maturity, we studied two near isogenic lines, *Gossypium hirsutum*, Texas Marker-1 wild type (TM-1) and immature fiber (*im*) mutant showing a significant difference in MIC values. The fibers from *im* mutant plants were finer and less mature with lower MIC values than those from the recurrent parent, TM-1. A comprehensive fiber property analysis of TM-1 and *im* mutant showed that the lower MIC of fibers in *im* mutant was due to the lower degree of fiber cell wall thickening as compared to the TM-1 fibers. Using an F_2 population comprising 366 progenies derived from a cross between TM-1 and *im* mutant, we confirmed that the immature fiber phenotype present in a mutant plant was controlled by one single recessive gene *im*. Furthermore, we identified 13 simple sequence repeat markers that were closely linked to the *im* gene located on chromosome 3. Molecular markers associated with the *im* gene will lay the foundation to further investigate genetic information required for improving cotton fiber fineness and maturity.

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Introduction

Cotton (*Gossypium* sp.) is the world's most important natural fiber. Although the consumption of cotton fibers has been steadily increasing during the last two decades, weak demand for natural fibers is expected to continue due to a strong competition from man-made fibers in the highly competitive global textile market (Ethridge 2010). To stay competitive, cotton breeders have been attempting to improve cotton fiber properties that greatly affect the quality of yarns. Among the cotton fiber properties, fiber fineness and maturity have been considered as very important traits affecting yarn quality because the number of fibers in the cross-section of a yarn is limited by fiber fineness and maturity. These properties directly affect yarn strength and performance. Therefore, fiber grade and price in the cotton fiber market are determined by the value of fiber fineness and maturity that are indirectly measured as "micronaire" by determining the air-flow resistance through a plug of cotton fibers of a given weight.

Despite the importance of fiber fineness and maturity, the genetic control and molecular mechanisms that regulate these fiber properties have not been well characterized. In early 1970s, an immature fiber (*im*) mutant that had immature fiber phenotype was identified in Upland cotton (*Gossypium hirsutum* L.) variety Acala 4-42 (Kohel et al. 1974). The cotton bolls of *im* mutant plants were non-fluffy, and appeared as not mature (Fig. 1). The micronaire (MIC) value of mutant fiber was low, sometimes too low to measure using a standard High-Volume Instrumentation (HVI) instrument. Fiber elongation between TM-1

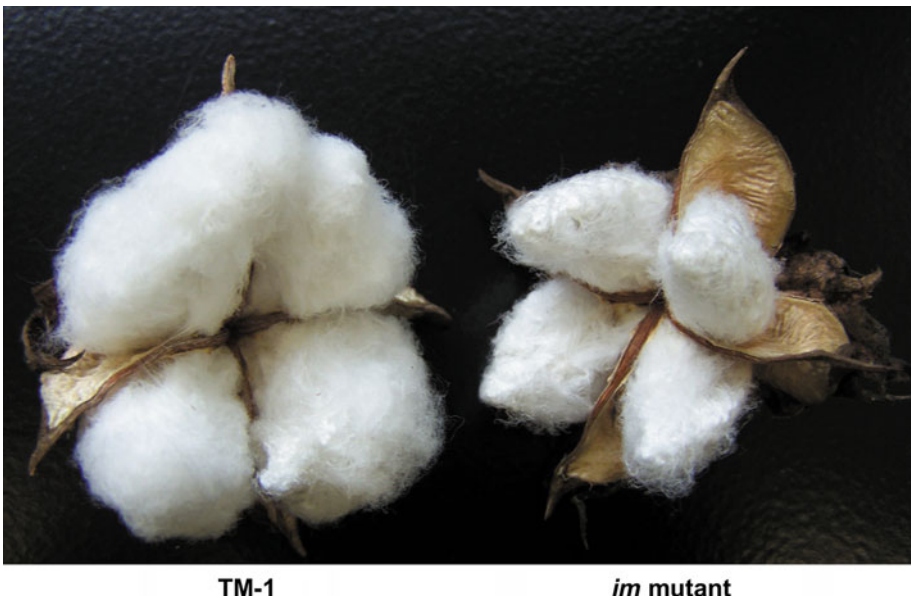

Fig. 1 Phenotypes of two cotton NILs showing different micronaire. *G. hirsutum* Texas Marker-1 (TM-1) with a high micronaire showed fluffy bolls, whereas immature fiber (*im*) mutant with a low micronaire showed non-fluffy bolls

(a genetic standard of Upland cotton) and the *im* mutant was similar, whereas total dry weight of mutant fibers was reduced as much as 40 % when compared with TM-1 (Kohel et al. 1974). Fiber properties measured by HVI showed that fiber length and strength of mutant fibers were also reduced (Kohel and McMichael 1990). The mutant was crossed to TM-1, and backcrossed four times using TM-1 as recurrent parent to obtain a near isogenic line in TM-1 background (Kohel and McMichael 1990). Based on the segregation results of the F_2 progeny, the non-fluffy phenotype in the mutant was controlled by one single recessive gene designated *im* (Kohel and McMichael 1990). Later, a cytogenetic study using hypoaneuploid cottons showed that the *im* gene was associated with chromosome 3 (Kohel et al. 2002).

Although the *im* mutant was discovered about 40 years ago, and it appeared to have provided a novel and unique opportunity to identify genetic information underlying cotton fiber fineness and maturity, the *im* mutant has not been well studied partially due to difficulties of distinguishing the phenotypes between TM-1 and the mutant (Kohel and McMichael 1990). The non-fluffy phenotype of the *im* mutant was greatly affected by adverse weather conditions like strong wind, high humidity or rain. The wild-type TM-1 cotton plants often produced an *im* mutant-like phenotype, referring tight lock bolls, under stress conditions caused by drought, cold temperature, and pathogens (Kohel and McMichael 1990). Thus, molecular markers associated with the *im* gene have not been identified yet.

We re-examined fiber properties of TM-1 and *im* mutant using image analyses, gravimetric fineness in addition to the classic fiber measuring technologies like HVI and Advanced Fiber Information System (AFIS). We found that the MIC value difference between TM-1 and *im* mutant fibers was due to the difference of cell wall thickening. We also found that lint percentage (lint %) was a simple and quick determinant of differentiating *im* mutant from TM-1 regardless of growing locations and environmental conditions. In this research, we made a cross between TM-1 and *im* mutant, measured lint % and fiber MIC value as well as gravimetric fineness of each F_2 progeny. Then, we identified molecular markers linked to the *im* gene. The results reported herein will help in identifying the genes or gene-networks regulating fiber fineness and maturity, which, in turn, could be used to improve cotton fiber quality traits through breeding or biotechnology.

Materials and methods

Plant materials

Two cotton near isogenic lines (NILs), *G. hirsutum* Texas Marker-1 (TM-1) and immature fiber (*im*) mutant were kindly provided by Dr. Russell Kohel at USDA-ARS, College Station, TX. These NILs were developed as described before (Kohel and McMichael 1990). Both NILs were grown in a field and greenhouse of USDA-ARS in New Orleans, LA from 2007 to 2011, and in a field in Stoneville, MS in 2011. The cotton plants directly affected by the Hurricane Gustave in 2008 were not used for fiber property measurements due to a high frequency of abnormal (tight lock) phenotype in TM-1.

A cross was made between TM-1 and *im* mutant plants using TM-1 as female in a greenhouse in 2010. Five F_1

plants were self-pollinated to obtain F_2 seeds. F_2 plants along with parents were grown in a field in Stoneville, MS in 2011. Standard conventional field practices were applied during planting season. Phenotypes of the segregating F_2 population were evaluated for lint %, MIC, and gravimetric fineness. Leaf samples were collected from 366 F_2 plants as well as parents for DNA isolation.

The soil type in Stoneville, MS was Bosket fine sandy loam. The soil type in New Orleans was Aquent dredged over alluvium in an elevated location to provide adequate drainage. The soil used in greenhouse was Metro-Mix 350 from Hummert International (Earth City, MO).

Fiber property measurements

All dried and mature fibers from the two NILs were ginned using a laboratory roller gin. Lint % was measured by dividing the seed cotton weight with lint weight, and multiplying by 100. For the measurement of fiber properties, fibers were pre-equilibrated with 65 % humidity at 21 °C for 48 h. The average fiber properties of five tests were determined by the Cotton Fiber Testing Lab in USDA-ARS-SRRC located in New Orleans, LA. Upper-half mean fiber length (UHML), uniformity, bundle strength, and MIC were measured by HVI (USTER Technologies Inc., Charlotte, NC) that is the standard method to obtain cotton fiber quality data measurements by USDA. The Fibronaire instrument (Motion Control Inc., Dallas, TX) was also used to measure MIC values for a few F_2 progenies that did not produce enough fiber for HVI measurement. Upper quartile length (UQL), mean fiber length (Lw), fineness, and maturity ratio were measured using an AFIS (USTER Technologies Inc.). UQL and Lw were expressed on a weight basis. In AFIS, fineness and maturity ratio were estimated from individualized fibers using an optical technology. Gravimetric fiber fineness was directly measured as mass per unit length, and reported as millitex (mtex) that is milligrams per kilometer of fibers. The weight of 300 fibers that were combed and cut at the top and bottom to leave 15 mm long was measured by a microbalance. Average fiber fineness was calculated from three tests.

Image analysis to measure fiber maturity

The fiber samples were embedded, thin-section cut, and photographed using the method previously described (Boylston et al. 1993). The image analysis system was a Leica Model 600 interfaced to a Nikon Optiphot POL light microscope operating in the transmitted mode. The wall area (A) excluding lumen and perimeter (P) of the fiber cross sections was measured according to Thibodeaux and Rajasekaran (1999). The circularity (θ) or degree of thickening was calculated using the equation, $\theta = 4\pi A/P^2$.

Bulked segregant analysis (BSA)

Young leaves were collected from each individual F_2 plants and parents, and stored at −80 °C. Total DNA was extracted from frozen leaves according to Fang et al. (2010). To rapidly identify DNA markers associated with the *im* gene, simple sequence repeat (SSR) markers were first screened for polymorphism between two parents, TM-1 and the *im* mutant. Then, these polymorphic markers were screened using the BSA method (Michelmore et al. 1991). For the wild type (TM-1 type) bulk, DNA of ten F_2 plants whose lint % and MIC value were higher than 35 % and 4.0, respectively, were pooled at equal ratio and diluted to 50 ng/μL. The mutant type bulk consisted of DNA from ten F_2 plants whose lint % and MIC value were lower than 20 % and 2.6, respectively. We made four DNA bulks, two for each type. Only SSR markers that revealed consistent polymorphism between two pairs of DNA bulks were analyzed among the 366 F_2 progeny plants.

SSR marker analysis

The DNAs of TM-1 and *im* mutant parents were analyzed with 2,183 SSR markers. Most of these markers were mapped in the TM-1 × *G. barbadense* 3-79 high-density map (Yu et al. 2012). After BSA analysis, a great majority of the markers showing consistent difference between two pairs of DNA bulks were mapped on chromosome 3 (Chr.03). Thus, we carefully checked five high-density genetic maps (Guo et al. 2008; Lacape et al. 2009; Xiao et al. 2009; Yu et al. 2011, 2012), and selected SSR markers mapped on Chr.03 that we did not include in the first round of screening. Because the homeologous chromosome of Chr.03 was suggested as Chr.14 (Yu et al. 2012) or Chr.17 (Guo et al. 2008; Lacape et al. 2009; Rong et al. 2004), we also selected markers from both Chr.14 and Chr.17. Altogether, we selected 161 new SSR markers mapped in one of these three chromosomes in at least one of the five maps. We synthesized new oligos, and analyzed them using two pairs of DNA bulks as PCR templates. Primer sequences of SSR markers can be obtained from Cotton Marker database (http://www.cottonmarker.org) and CottonGen database (cottongen.org). The primer sequences of the SSR markers associated with the *im* gene reported here are also listed in Table 1. Forward primers were fluorescently labeled at 5′ end with 6-FAM (6-carboxyfluorescein), HEX (4,7,2′,4′,5,7-hexachloro-carboxyfluorescein) or NED (7′,8′-benzo-5-fluoro 2′,4,7,-trichloro-5-carboxyfluorescein). SSR primers were purchased from Sigma Genosys (Woodlands, TX) or Life Technologies (Foster City, CA). Multiplex PCR was performed when conducting primer screening. Three pairs of primers with different dyes were multiplexed in each PCR reaction.

Table 1 Primer sequences and allele sizes of thirteen SSR markers associated with the *im* gene

#	Marker	Forward sequence 5′ ≫ 3′	Reverse sequence 5′ ≫ 3′	Allele sizes (bp) in TM-1 and *im* mutant
1	BNL0244	AGATTGAAATGCAGCTTCGG	TTTGGAAGAGCACAAAACCC	201 and 199
2	BNL4034	GAGGAAGGATGGCTAACTGGTATAA	GTCAAAACACATATCACACACACAC	85 and 83
3	CGR5871	TTACCGGGTCTGGGATATTG	ATGCAGCTTCGGAATCTCTG	115 and 113
4	DPL0170	AGCACAAGAAAGAAGGAAGGAAG	GCTCAAAGACCTGAGTTTGATTCT	231 and 237
5	DPL0733	GGCTACTATGGTGTTCGTTTCAAT	GTATCGCCTTGATGCAGAAACTAC	192 and 190
6	DPL1071	GAGAAAAACCACAAGGCCCTA	GGAAACCCACCCAATTTAAGA	290 and 293
7	MUSS172	TTTCTATCCCACCATTTTCCC	GTGTTTGTGTCACTGTGCCG	201 and 221
8	NAU1190	CCATGTCCGTATCCATGTTA	TAAGGCAAGATAGGGTCAGG	213 and 233
9	SHIN-0313	AGCAAGATAACCCATGTCCG	GTTTGGGAGGGTGGTGAGTA	110 and 131
10	SHIN-1511	TTCTGAAGGGACTGAAAGCG	AATGGCCGTTGATCATGTG	179 and 177
11	TMB1898	TTGGTTCATATTGGTTCGGTTA	AACCACTGTTCCGACCTCAC	219 and 217
12	TMB1963	CCCACCAGCATATGATGAAA	GCATGCAGTGAGCGTGAGTA	217 and 206
13	UCD540	TTTCAAGGAGCCAAATGCTT	TGTGATCGAAACAGCACCAT	294 and 292

After an SSR marker was putatively identified as linked to the trait, this marker was further analyzed using non-multiplex PCR. The PCR amplification conditions and marker data acquisition were according to Fang et al. (2010). If a marker revealed two segregating loci within the population, the duplicate marker loci were designated by adding a lower-case letter in alphabetical order after the primer name.

Linkage analysis

First, SSR markers from 366 F_2 progeny were mapped using program JoinMap3.0 (Van Ooijen and Voorrips 2001a) with LOD score ≥15.0. Second, the marker map was imported into the program MapQTL 4.0 (Van Ooijen and Voorrips 2001b), the lint % and MIC value of F_2 progeny were analyzed using MapQTL 4.0 to conduct quantitative trait locus (QTL) analysis and determine the genetic control of the immature fiber phenotype present in mutant line. Third, because the QTL analysis indicated that the immature fiber phenotype in the mutant plant was controlled by one single recessive gene, we arbitrarily classified the F_2 progeny into two types: wild type (lint % ≥26 %) and mutant type (lint % <26 %). Then the genomic location of the *im* gene was mapped using the program JoinMap 3.0.

Results

Comparison of fiber properties between TM-1 and mutant fibers

The phenotype difference between TM-1 and *im* mutant reported previously by Kohel's group (Kohel and McMichael 1990; Kohel et al. 1974) was only from College Station, TX. To test whether the phenotype difference was reproducible in other locations, we grew both TM-1 and *im* mutant line at two different locations (Louisiana and Mississippi) for multiple years (2007–2011). Although windy and rainy weather conditions could cause difficulties to differentiate between the true non-fluffy phenotype of the *im* mutant and non-fluffy type resulted from weather factors, the differences of lint % between TM-1 and mutant fibers were consistent regardless of growing locations and seasons (Fig. 2a). Despite the fluctuation of the lint % of TM-1 (35.0–41.2 %) and immature fiber mutant (17.7–29.0 %) between different years, the difference of the lint % between these two lines in the same season was quite consistent. The average lint % of *im* mutant was about 14.5 percentage points lower than that of TM-1 each year (Fig. 2a). The average lint % of the *im* mutant grown at a cotton field from Louisiana for 4 years was 22.7 %, whereas that of TM-1 was 37.2 %. The same trend was observed for the plants grown either in a greenhouse in Louisiana or in a field in Mississippi. The lint % of *im* mutant was consistently 10–15 percentage points lower than that of TM-1 (Fig. 2a).

Fiber property measurements obtained by HVI showed that the MIC value of *im* mutant fibers was 37 % lower than that of TM-1 fibers (Fig. 2b). Little differences of fiber length (UHML) and uniformity were detected between TM-1 and mutant line, whereas a detectable reduction (7.2 %) of fiber strength was observed in *im* mutant fibers as compared to TM-1 fibers (Fig. 2b).

The comparisons of fiber properties measured by AFIS showed little difference in fiber length (UQL and Lw) between two NILs, but statistically significant reductions of fineness (5.9 %) and maturity ratio (5.0 %) of *im* mutant fibers (Fig. 2c). The low MIC value and maturity ratio of

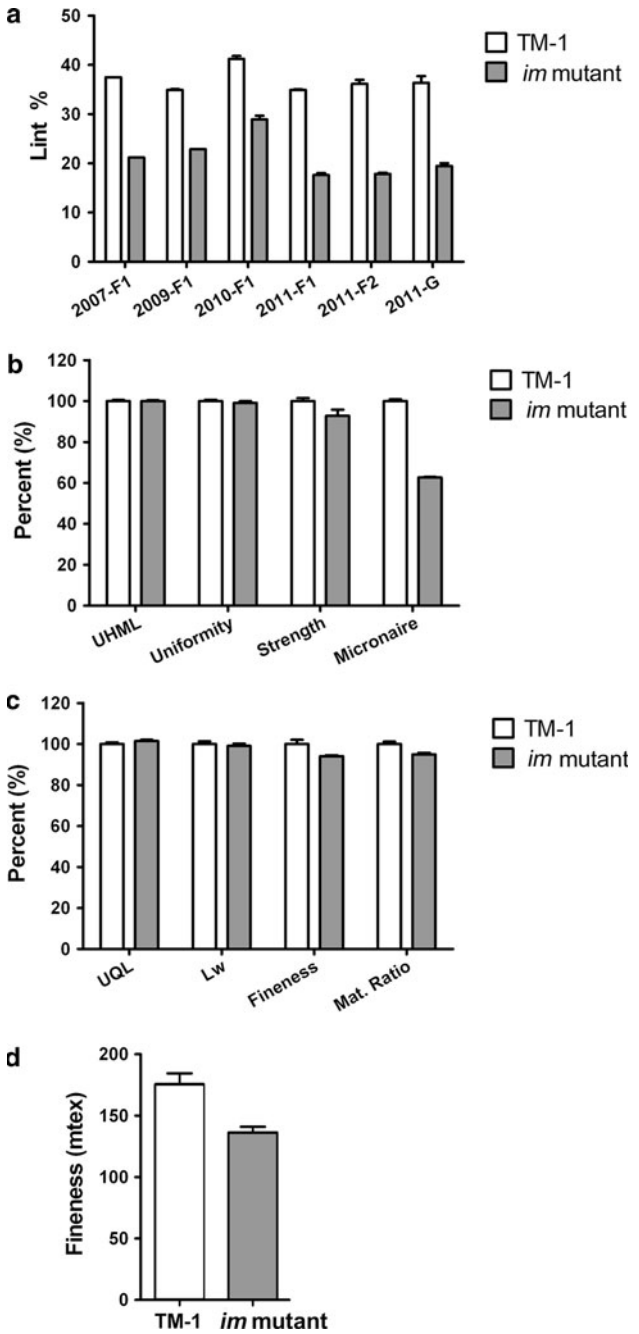

Fig. 2 Comparisons of fiber properties between TM-1 and *im* mutant. **a** Measurements of lint % of TM-1 and *im* mutant grown in a field of New Orleans, LA (F1) and Stoneville, MS (F2) or a greenhouse (G) of New Orleans, LA between year 2007 and year 2011. **b** Comparison of fiber properties between TM-1 and *im* mutant fibers harvested in 2007 and measured by HVI. The ratio of values of fiber length (UHML), uniformity, fiber strength, and micronaire from *im* mutant fibers were presented as percent of TM-1 fibers. **c** Comparison of fiber properties between TM-1 and *im* mutant fibers harvested in 2007 and measured by AFIS. The ratio of values of fiber length (UQL and Lw), fineness, and maturity ratio from *im* mutant fibers were presented as percent of TM-1 fibers. **d** Comparison of gravimetric fineness between TM-1 and *im* mutant fibers harvested in 2007

im mutant fibers indicated that mutant fibers were less mature than TM-1 fibers. Fiber maturity refers to mean degree of fiber cell wall thickening relative to the perimeter of effective diameter of the fiber. We measured gravimetric fineness of fibers that is directly proportional to the cell wall area. Figure 2d showed that the value of gravimetric fineness from *im* mutant fibers (136.3 mtex) was 22.4 % lower than that from TM-1 (175.6 mtex). The result shown in Fig. 2d implied that the lower MIC value of the *im* mutant fibers was resulted from the thinner cell wall as compared to the TM-1 fibers.

Image analysis to measure fiber maturity

All fiber property results measured by HVI, AFIS, and gravimetric fineness test supported a hypothesis that *im* mutant fibers had lower degree of cell wall thickening than TM-1 fibers. To confirm this, the fiber bundles of both lines were thin sectioned, and photographed (Fig. 3a). The differences of fiber maturity (degree of fiber cell wall thickening) between two NILs were determined by microscopic image analyses of thin sections of fiber bundles (Fig. 3a, b). Consistent with the results of gravimetric fineness implying lower cell wall area of *im* mutant fibers than TM-1 fibers, the cell wall area of most cross-sectioned *im* mutant fibers was thinner than that of TM-1 fibers (Fig. 3a). The cell wall area (A) and perimeters (P) of individual fiber sections from multiple microscopic images were measured to determine the circularity (θ) referring degree of fiber cell wall thickening. There was a very little but detectable difference (4.56 %) of the average perimeter size of individual fiber between *im* mutant (51.5 μm) and TM-1 (53.8 μm) (Fig. 3b), but there was significant difference (37.7 %) of average cell wall area excluding lumen (vacuole) area between *im* mutant (70.1 μm^2) and TM-1 (112.4 μm^2) (Fig. 3b). The circularity (θ) of *im* mutant fibers (0.37) was 26 % lower than that of TM-1 fibers (0.50) (Fig. 3b). Based on these results, we concluded that the non-fluffy phenotype in *im* mutant was caused by the low degree of fiber cell wall thickening.

Inheritance of immature fiber phenotype in *im* mutant

Kohel and McMichael (1990) reported that the immature fiber mutation was controlled by one single recessive gene *im*. In the present study, we used a single large F_2 population to validate this determination. Before harvesting bolls from each F_2 progeny grown in Stoneville, MS, we did a preliminary count based on the phenotypes of bolls (Fig. 1). Roughly about 25 % progeny had non-fluffy boll. Then, we harvested cotton bolls, and obtained lint % and

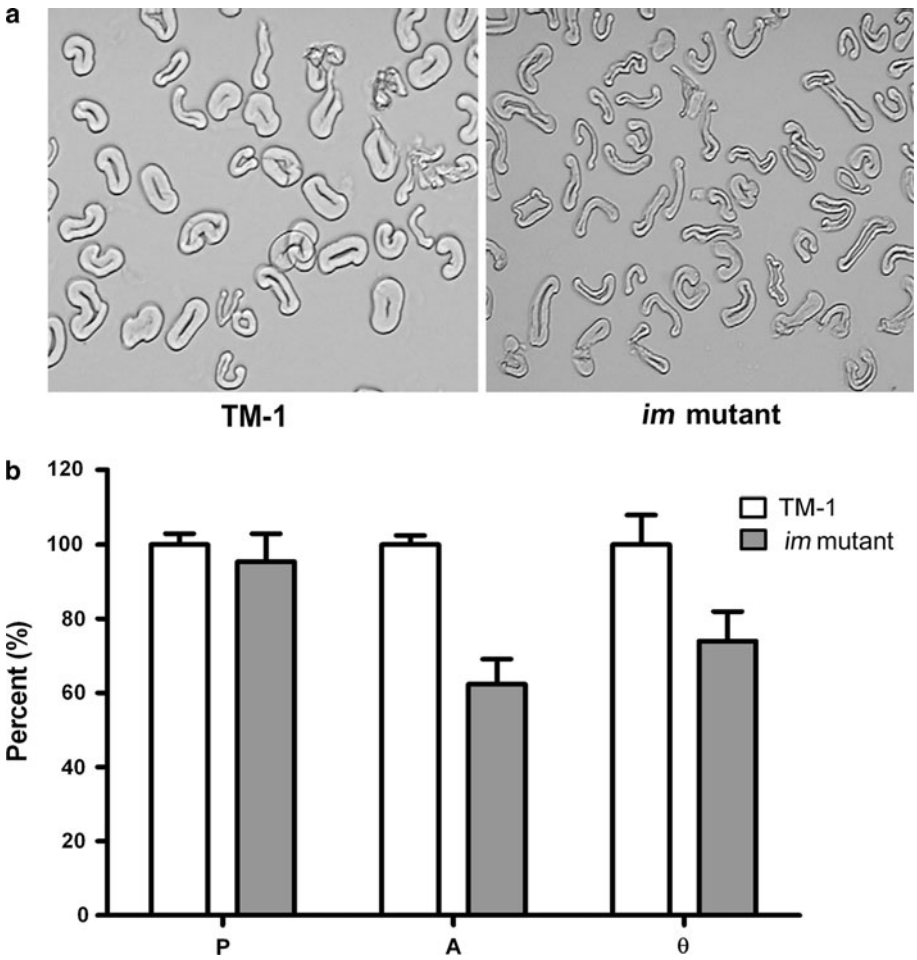

Fig. 3 Image analyses of fibers from TM-1 and *im* mutant. **a** Microscopic image analyses of cross-sectioned fibers from TM-1 and *im* mutant. **b** Comparison of fiber maturity (degree of cell wall thickening) between TM-1 and *im* mutant. Fiber perimeter (*P*), cell wall area (*A*), and circularity ($\theta = 4\pi A/P^2$)

fiber MIC value for 351 F_2 progeny plants. No cotton fibers were harvested from 15 F_2 plants that failed to produce enough bolls. The lint % of F_2 progeny ranged from 11.72 to 54.32 % with an average of 30.24 %. The lint % of the parents grown in the same field was 33.8 and 18.6 % for TM-1 and *im* mutant, respectively. Although a little arbitrarily, we divided the F_2 plants into two types: wild type and mutant type using the lint % of 26 % (a mid-point between the averages of two parents) as a threshold. Two hundred sixty-eight F_2 plants had lint % higher than 26 %, and the average was 33.7 %. They were classified as wild type. Eighty-three F_2 plants had lint % lower than 26 %, and the average was 18.95 %, and they were classified as mutant type. The ratio fit the expected 3:1 segregation ratio ($\chi^2 = 0.34$). The fiber MIC values of the F_2 plants ranged from 2.4 to 6.0 with an average of 4.42. The average MIC value of TM-1 was 4.5 but that of *im* mutant was not calculated because many of them had MIC value below the HVI detection limit (<2.4). The fiber MIC values of F_2 plants were very well correlated with lint %. The 268 F_2 plants classified as wild type had an average of MIC value of 4.52 (between 2.5 and 6.00) while the average MIC value of 83 mutant type F_2 plants was 2.61 (between 2.4 and 4.4) (the value 2.4 was used for plants whose MIC were below detectable level by HVI). Our results supported that the non-fluffy phenotype present in *im* mutant was controlled by one single recessive gene.

Identification of SSR markers associated with *im* gene

Of the 2,183 SSR markers screened, 466 (21.3 %) were polymorphic between the parents TM-1 and *im* mutant line. After evaluating these 466 markers using 4 DNA bulks as PCR templates, 12 markers showed consistent polymorphism between two pairs of DNA bulks. Of the 12 markers, 8 were mapped on Chr.03, 2 on Chr.14, 1 on Chr.07, and 1 unmapped in the interspecific *G. hirsutum* TM-1 × *G. barbadense* 3-79 map (Yu et al. 2012). Because Chr.14 was considered as the homeologous chromosome of Chr.03 (Yu et al. 2012), it was not a surprise that markers previously mapped on Chr.14 were potentially associated with the *im* gene. Based on the BSA results, it was clear that the *im* gene was residing in Chr.03. To identify more markers associated with the *im* gene, we examined other four high-density maps (Guo et al. 2008; Lacape et al. 2009; Xiao et al. 2009; Yu et al. 2011) in

addition to the map of Yu et al. (2012). An additional 161 markers that were mapped either on Chr.03, Chr.14 or Chr.17 in at least one of the maps were selected. We analyzed these 161 new markers using the 4 DNA bulks as PCR templates. Only two markers, NAU1190 mapped on Chr.03 by Guo et al. (2008) and CGR5871 mapped on Chr.14 by Xiao et al. (2009), revealed polymorphism between DNA bulks.

All together, 14 markers identified by BSA were used to genotype 366 F_2 progeny. Three markers, BNL0244, CGR5871 and UCD540 revealed two segregating loci each among the progeny. The other 11 markers generated only one segregating locus each within the population. A total of 17 loci were scored and used for map construction. Thirteen marker loci (Fig. 4) were mapped within a region of 4.3 cM. SHIN-0183 that was previously mapped on Chr.07 was not mapped, a confirmation of false positive resulting from BSA. The second locus revealed by BNL0244, CGR5871 or UCD540 was not mapped together with the large group of 13 loci.

As mentioned earlier, our criterion to classify wild and mutant type based on the lint percentage value of 26 % was a little arbitrary. To further confirm the inheritance of single recessive gene, we conducted QTL analysis using the program MapQTL 4.0. We used both lint %, and MIC value. The composite interval QTL mapping result for lint % is shown in Fig. 5. The result of QTL analysis for fiber MIC value was similar to that for lint %. With LOD score as high as 95, our results clearly indicated that the lint % or MIC value of the F_2 progeny was not controlled by multi-genes, instead by one single recessive gene. The gene should be located around the region between the markers DPL1071 and SHIN-1511. A further map construction using JoinMap 3.0 indicated that the *im* genetic locus was flanked by markers DPL1071 and TMB1898 at a distance of 0.32 and 0.13 cM, respectively (Fig. 4).

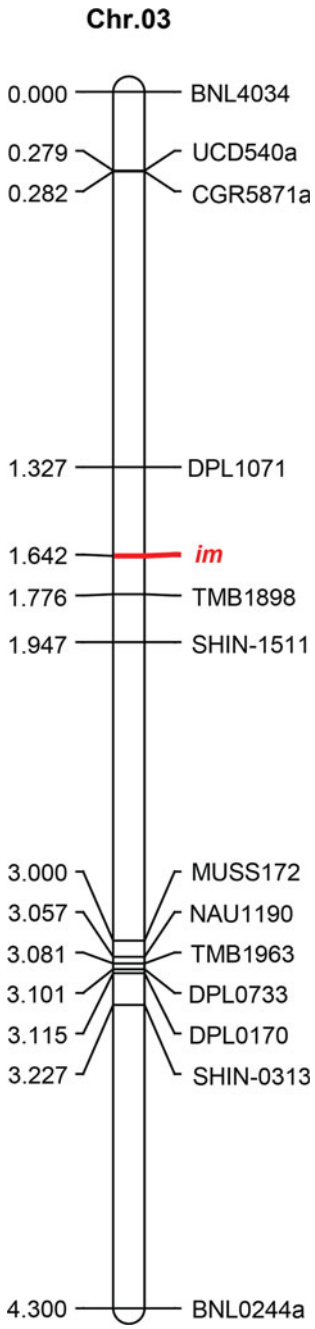

Fig. 4 Molecular markers associated with the *im* gene on chromosome 3

Discussion

Our comprehensive fiber property analysis showed that the average degree of fiber cell wall thickening from *im* mutant fibers was lower than that from TM-1 fibers. The lower degree of cell wall thickening caused the non-fluffy phenotype of *im* mutant fibers (Fig. 1) as reported by Kohel and McMichael (1990). The results from HVI, AFIS, gravimetric fineness, and circularity values consistently showed that the smaller cell wall area of the cross-sectioned *im* mutant fibers caused finer, weaker, and less mature fibers in *im* mutant as compared to the TM-1 fibers (Figs. 2, 3). Unlike the report by Kohel and McMichael (1990) that the average length of *im* mutant fibers (2.34 cm) was shorter than that of TM-1 (2.82 cm), our results showed little difference between TM-1 and *im* mutant fibers (Fig. 2) in fiber lengths that were measured by three different methods (UHML, UQL, and Lw). The difference between our results and those observed by Kohel and McMichael (1990) might be due to different ginning methods used. The shorter average length of *im* mutant fibers from the previous report (Kohel and McMichael 1990) might be resulted from a harsh ginning process with a saw gin that might have caused more damage to the weak and immature fibers of *im* mutant than to the strong and

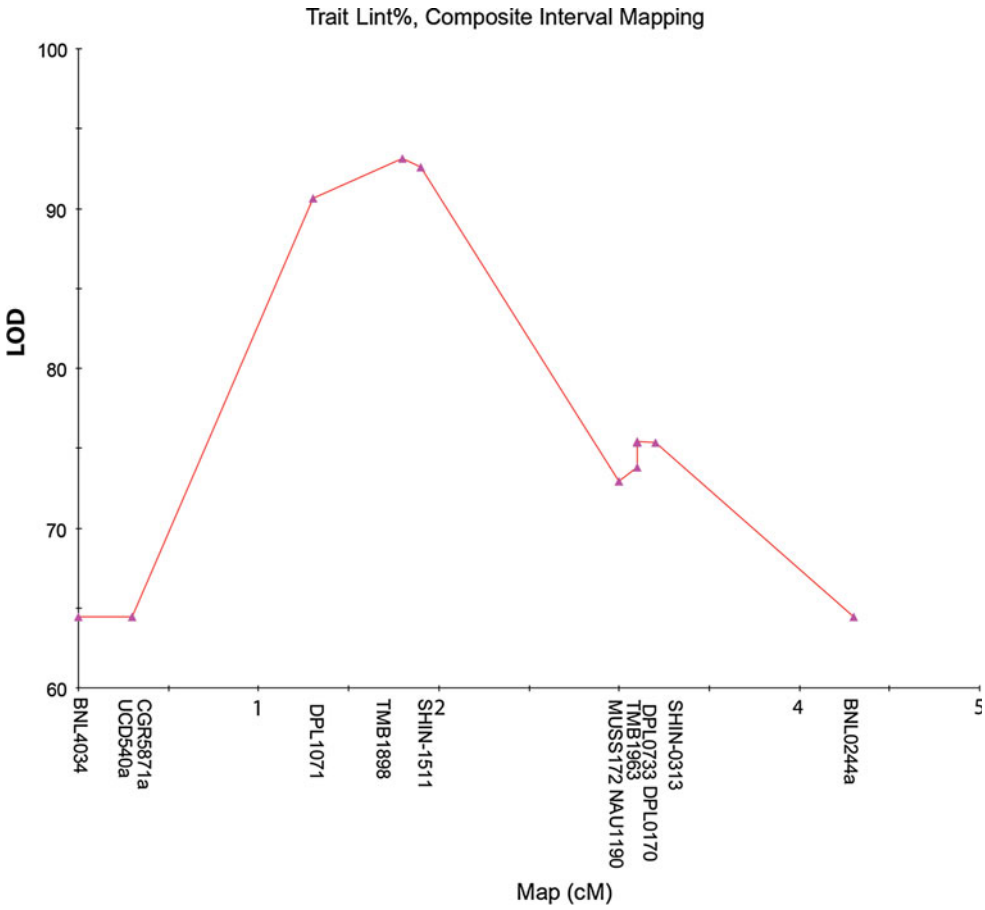

Fig. 5 The composite interval QTL mapping for lint %

mature TM-1 fibers. In the present study, we used a roller gin that was gentler to the fiber than a saw gin did. The significant difference of maturity, but little difference of fiber length between two NILs made the *im* mutant a suitable system for studying molecular mechanisms involved in cotton fiber maturity.

Despite the striking difference of MIC values between TM-1 and *im* mutant fibers, it was difficult to use MIC values for QTL analyses because *im* mutant fibers showing <2.4 of MIC could not be accurately measured using the current instruments like Fibronaire or HVI. The MIC values of *im* mutant fibers fluctuated around 2.4 or below from year to year. The average MIC of *im* mutant fibers in 2007 was a little above 2.4 as measured by HVI, whereas the MIC values of some F_2 progenies grown in 2011 could not be measured quantitatively because they were lower than 2.4. Different environments such as temperature and water availability in cotton plants in different growing seasons can significantly modulate the MIC values by affecting the rates of photosynthesis and cellulose biosynthesis (Bradow and Davidonis 2000). In an attempt to find a way to measure *im* mutant fibers quantitatively, we first tested if the lint % could be used as a determinant for classifying *im* mutant fiber. Our results showed that the lint % was not only a quantitative way to measure fiber property, but also a simple way to classify *im* mutant fibers accurately regardless of environmental conditions (Fig. 2a). Thus, the lint % instead of MIC was primarily used for analyzing F_2 progeny and QTL analysis (Figs. 4, 5).

The *im* mutant line used in the present study was developed by crossing the original mutant to TM-1, and then backcrossing four times using TM-1 as recurrent parent (Kohel and McMichael 1990). It supposed to be near isogenic to TM-1. However, our marker analysis showed much difference between them. It should be pointed out that most of the SSR markers used in this study were pre-screened for polymorphism between *G. hirsutum* and *G. barbadense*, and mapped. Thus, it was expected that the polymorphism between TM-1 and the *im* mutant line as revealed by these selected markers should be higher than that if revealed by randomly selected markers. But the 21.3 % polymorphism rate was exceptionally high for a pair of presumed NILs. Moreover, the polymorphism occurred in almost entire genome after examining the distribution of the polymorphic markers. It is not clear to us why the difference was so big. The original *im* mutant donor was an Acala cotton that was very different from TM-1, a Mississippi delta type cotton (Kohel et al. 1970).

The homeologous chromosome of Chr.03 was suggested as Chr.17 (Guo et al. 2008; Lacape et al. 2009; Rong et al. 2004; Xiao et al. 2009). Recently, Yu et al. (2012) suggested Chr.14 as more appropriate based on the centromeric cores between these three chromosomes. In the present study, our BSA analysis identified three markers that were previously mapped on Chr.14, but none from Chr.17. Because tetraploid cotton genome contains two subgenomes (At and Dt), it is very common to see the same marker be mapped in different but homeologous chromosomes by different research groups. There were many such examples among the six high-density cotton genetic maps (Guo et al. 2008; Lacape et al. 2009; Xiao et al. 2009; Yu et al. 2007, 2011, 2012). However, it was far less often to map the same marker in different and non-homeologous chromosomes. Our results did support Chr.14 as the homeologous chromosome of Chr.03 as suggested by Yu et al. (2012).

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