Development of SCAR Marker Linked to a Major QTL for High Fiber Strength and Its Usage in Molecular-Marker Assisted Selection in Upland Cotton

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ABSTRACT

Cotton fiber is an important raw material for the textile industry. As a result of the recent advances in the textile industry in spinning technology, greater cotton fiber quality, especially strength, is required (Deussen, 1992). Because of the rigors of gininning, opening, cleaning, carding, combing, and drafting, an important breeding objective has been to increase fiber strength. Fiber strength in Upland cotton grown in the USA has been increased by 0.19 cN tex\(^{-1}\) yr\(^{-1}\) over the 10-yr period of 1982 to 1992. The average strength of the commercial cultivars in 1991 to 1992 was close to 21.7 cN tex\(^{-1}\) (Benedict et al., 1999). There is an urgent need to improve cotton fiber quality further. However, there is no rapid way to do this by current plant breeding methods and on the basis of current genetic information. In traditional breeding to develop superior quality fiber properties, the quality of the fiber can be determined only after harvesting and testing the fiber. As a result, it is difficult, expensive, and time-consuming to develop cotton cultivars with superior quality fiber by these methods.

Advances in the use of DNA markers for MAS provide a promising method of streamlining plant breeding programs (Kohel et al., 2001; Lawson et al., 1997; Mohan et al., 1997). In 1999-2000, we used a *G. anomalum* Wawra ex Wawra & Peyritch introgression line 7235, which had been determined to possess good fiber quality, to identify molecular markers linked to fiber strength QTLs. By use of F\(_2\) and F\(_3\) populations derived from a cross between 7235 and TM-1, a widely used genetic standard of Upland cottons, a major QTL for fiber strength was identified. Eight molecular markers, two simple sequence repeat (SSRs) and six random amplified polymorphic DNA (RAPDs) markers, were linked to the QTL. The objective of this study was to develop rapid and reliable sequence characterized amplified region (SCAR) markers linked to the QTL for marker-assisted selection (MAS). Two RAPD markers, UBC4311920 and UBC7571365, were converted to SCAR markers after sequencing the two ends of the two polymorphic DNA fragments. Only SCAR4311920 marker detected polymorphism between TM-1 and 7235, whereas SCAR7571365 showed monomorphism. SCAR4311920 marker was explored to determine its stability in (7235 × TM-1) \(F_2\), with UBC4311920 marker as a control and to screen the major fiber strength QTL of (7235 × Simian 3) BC\(_1\)F\(_1\) population for transferring good fiber quality. The result showed that the specific SCAR4311920 marker could be applied to large-scale screening for the presence or absence of the major fiber strength QTL in breeding populations.

MATERIALS AND METHODS

Plant Materials

*Gossypium anomalum* introgression germplasm lines were developed by crossing *G. anomalum* with *G. hirsutum*, and then backcrossing to cultivars and strains with high fiber strength, such as Acala 3080, PD 8619, and PD 4381. The germplasm line 7235 was developed from a backcrossed prog-

**Abbreviations:** MAS, marker-assisted selection; QTL, quantitative trait locus; RAPD, random amplified polymorphic DNA; SCAR, sequence characterized amplified region; SSR, simple sequence repeat.
eny with Acala 3080 (Qian et al., 1992). The fiber strength of 7235 was 27.3 cN tex\(^{-1}\), its fiber length was 35 mm, and its fineness was 4.1 Micronaire units, and it was grown at Nanjing in 1998. TM-1, the genetic standard line for Upland cotton (Kohel et al., 1970), was obtained from USDA ARS, Southern Plains Agriculture Research Center, College Station, TX, USA. Its fiber strength was 20.7 cN tex\(^{-1}\), its length was 30.5 mm, its fiber fineness was 5.0 Micronaire units, and it was grown at Nanjing in 1998.

The same (7235 × TM-1) F\(_1\) population that was used for the tagging QTLs for fiber qualities (Zhang et al., 2003) was also used to identify the consistency of the objective marker after using UBC431\(_{1920}\) and SCAR431\(_{1920}\) to screen separately. To transfer the superior fiber quality gene from 7235 to commercial cultivars, Simian 3 (SM 3) in Yantze River valley was crossed with 7235. From (7235 × SM 3) BC\(_1\)F\(_2\), individual plants were selected on the basis of fiber quality and strength results. Individual plants selected from (7235 × SM 3) BC\(_1\)F\(_2\) were planted into families at the Jiangpu Breeding Station and Nanjing Agricultural University in 2000. Individual plants that were used to test the genetic stability of the QTL and its MAS result ranged from 5 to 8 in each family. All the cotton fiber from each individual plant from (7235 × SM 3) BC\(_1\)F\(_2\) was harvested for the fiber tests. Fiber samples were tested by Supervision, Inspection & Test Center of Cotton Quality, the Ministry of Agriculture, China. The difference in the fiber quality of the individual plants with and without the molecular marker in this advanced backcrossing/selfing population was determined by the t test.

**DNA Analysis**

Total DNA was extracted from a healthy leaf of each of the (TM-1 × 7235) F\(_1\) plants in the breeding population by the cetyltrimethylammonium bromide (CTAB) method (Paterson et al., 1993) with minor modifications in the elimination of diethyldithiocarbamic acid. Following a single chloroform-isoamyl alcohol (24:1) extraction, the precipitated DNA was resuspended in sterile TE for amplification by PCR.

All PCR amplifications were performed in 20-μL volumes in a Perkin-Elmer 9600 Thermocycler (Foster City, CA). RAPD experiments included 45 cycles of amplification (at 95°C for 30 s, at 40°C for 1 min, at 72°C for 1.5 min after an initial denaturing step at 95°C for 2 min), performed in a 25-μL reaction volume containing 0.5 units Taq DNA polymerase (Sigma, St. Louis, MO), a buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), a 0.5 μM primer, 100 μM dNTPs (dATP, dTTP, dGTP, dCTP), and 3.5 mM of MgCl\(_2\) supplied by the enzyme manufacturer. PCR products were separated in 1.4% (w/v) agarose gel and visualized under UV light after ethidium bromide staining. High Fiber Strength QTLs

**RESULTS**

**Cloning and Sequencing of UBC431\(_{1920}\) and UBC757\(_{1365}\)**

To develop a reliable SCAR marker for the detection of UBC431\(_{1920}\) and UBC757\(_{1365}\), the polymorphic DNA fragments, with 1920- and 1365-bp molecular weights amplified from 7235, were cloned separately into the pGEM-T easy vector (Promega, Madison, WI, USA) after they were confirmed to be RAPD bands through running gel on 2% (w/v) agarose for each dilution as recommended by the manufacturer. The recombinant vectors were separately used to transform competent Escherichia coli DH5α cells. The different clones, p1365 and p1920, were sequenced on an ABI Prism 377 DNA Sequencer (PE Biosystems, Foster City, CA) after the correct insert size was confirmed by EcoRI digestion. Double-strand sequencing was done by means of the dideoxy-chain termination method with SP6 and T7 universal primers. The sequencing experiment was conducted by Shanghai Sangon Company.

**Development of SCAR Markers Linked to High Fiber Strength QTLs**

On the basis of the sequences of the two ends in p431-1920 and p757-1365, two pairs of 21 to 24 bases long (P1F and P1R, P2F and P2R), which included the original 10 bases of random primer of UBC431 and UBC757, respectively, were designed.

p431-1920F: CTGCGGGTCATGATCCTGAATA
p431-1920R: CTGCGGCTGAAGATATTACAG
p757-1365F: GGAAGGGAGGCGGTCATAAAGT
p757-1365R: GGAAGGGAGGAAACTATTG

Primer combination p757-1365F and p757-1365R amplified a 1365-bp DNA fragment not only in high strength parent 7235 and its F\(_1\), but also in TM-1, which suggested that the pair of primers could not be used in

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**REFERENCES**

assisted selection for high strength fiber QTLs. Primer combination p431-1920F and p431-1920R (designated as SCAR431) amplified a 1920-bp DNA fragment only in the donor parent 7235 and its F_{2}, but not in TM-1 (Fig. 2). To identify the consistency of amplification results by primers UBC431 and SCAR431, further investigation was conducted with the same (7235×TM-1) F_{2} segregation population as was used in the tagging of high strength fiber QTLs (Zhang et al., 2003). In a total of 90 RAPD reactions, 17 (18.9%) yielded no UBC431_{1920} products, while 73 (81.1%) had the expected result. SCAR431 primer combinations also amplified the same 90 individuals of the (7235×TM-1) F_{2} population; the results of producing targeted polymorphic bands were nearly 96% consistence with those that used UBC431 primer. The difference is that the products amplified with UBC431 primer were weak, unstable, and difficult to identify (Table 1).

**Evaluation of the RAPD, SSR, and SCAR Markers Linked to High Strength Fiber QTLs for Selecting High Strength Plants**

On the basis of our having crossed (SM 3×7235) BC_{1}F_{2} families for transferring high fiber strength QTLs, we selected five to eight individual plants from each family to test the advantage of the SCAR marker over the RAPD markers in the detection of high strength fiber QTLs. This was done to determine the genetic stability of the QTL and its MAS. A total of 164 high strength single plants from (SM 3×7235) BC_{1}F_{4} derivatives were tested for fiber quality by means of SCAR431_{1920} and were subjected to the analysis of two RAPD markers (UBC301_{431} and OPM07_{1047}) and one SSR marker (SSR1521_{130}) as a control. SCAR431_{1920} produced clearly targeted products in the breeding population (Fig. 3). Compared with the result derived from screening with two RAPD primers and one SSR marker (Table 2), the consistency rates, in terms of presence or absence of the targeted product, were 88.4% for UBC301_{431} and OPM07_{1047}, 92.1% for UBC301_{431} and SCAR431_{1920}, 96.3% for SCAR431_{1920} and OPM07_{1047}, and 92.1% for SCAR431_{1920} and SSR1521_{130}, respectively. Some crossovers occurred among these three markers. The genetic distance was 6.7 cM between UBC431_{1920} and OPM07_{1047}, 4.0 cM between UBC431_{1920} and SSR1521_{130}, and 8.9 cM between UBC431_{1920} and UBC301_{431}, the pair of markers with the most frequent crossovers. The result was consistent with the linkage map shown in Fig. 4. All the individuals having the marker showed high fiber strength. Fiber strength of SM 3, a commercial cultivar, was 22.00cN tex^{-1} in 2001.

**DISCUSSION**

Fiber strength is typically a quantitatively inherited trait (see review by May, 1999). Additive gene action predominates and five (Self and Henderson 1954) to as many as 14 (Tipton et al., 1964) genes were found to influence fiber strength. However, some data suggested that fiber strength might not always segregate in a quantitative manner, the inheritance analysis of some introgression lines particularly suggested this. Richmond (1951) indicated that recovery of high strength segregants from small backcross population during introgression of the triple hybrid G. thurberi Todaro × G. arbor-eum L. × G. hirsutum L. was evidence for only a few major genes controlling fiber strength. Meredith (1977, 1992) came to a similar conclusion. Two further backcrosses were made to Deltapline 16 nectarless to produce a nectarless strain, MD65-11, which had strength about 10% higher than that of Deltapline 16. MD 65-

Table 1. Comparison of UBC431_{1920} and SCAR431_{1920} markers for the detection of the high strength QTL in (7235×TM-1) F_{2} population.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Plants for PCR amplification</th>
<th>Number of amplified plants</th>
<th>Number of nonamplified plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBC431_{1920}</td>
<td>90</td>
<td>73 (81.1%)</td>
<td>17 (18.9%)</td>
</tr>
<tr>
<td>SCAR431_{1920}</td>
<td>90</td>
<td>76 (84.4%)</td>
<td>14 (15.5%)</td>
</tr>
</tbody>
</table>

Fig. 1. EcoRI digestion of the recombinant clones. Lane 1-2: EcoRI digested p757-1365; Lane 3: undigested p757-1365; Lane 4: EcoRI digested p431-1920; Lane 5: undigested p431-1920; and M: Lambda DNA/EcoRI + HindIII.
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Plate 1: M, 1-29; Plate 2: M, 30-58; Plate 3: M, 60-87

Fig. 3. The amplified results with SCAR4311920 in backcrossing/selfing breeding population M. Lambda DNA/EcoRI + HindIII. Lane 1-4 showed amplification product of 7235, TM-1, F1 and p431-1920; and lanes 5-87 represented different high strength individual plant from (SM 3 x 7235) BC1F2 families.

11 was crossed with Deltapine 90 followed by two backcrosses to Deltapine 90 with selection first for nectarless and then for bundle strength. Genetic analysis of variance components suggested that the 9% increase in strength could be conditioned by no more than a single gene. However, subsequent analysis with larger population sizes indicated that two major genes, which might be linked, were responsible for the high strength trait. From our genetic and molecular tagging results, we concluded that the higher fiber strength trait in 7235 was controlled by one major gene and a polygene (Yuan, 2000). Thus, it is feasible that selection by means of the high strength major QTL marker from 7235 will be successful.

Molecular markers are useful in plant breeding because the presence of targeted traits can be detected without waiting for the phenotypic expression of the gene (Ribaut et al., 1997). Furthermore, molecular markers can be used simultaneously to improve lint yield and fiber strength, which has become a major breeding consideration owing to a strong negative association between lint yield and fiber strength. In this study, SCAR4311920 could be used for selecting high strength individual in the segregating population. The genetic stability of a major QTL for fiber strength and its efficiency of MAS were studied in 164 progenies in BC2F2 generations of SM 3 x 7235 with 7235 as a parent with two RAPD markers, UBC301933 and OPM071047, and a SSR marker, with SSR1521130 as a control. The results revealed that the QTL for fiber strength associated with the four inherited markers steadily advanced in segregating generation populations. The mean difference of fiber strength between individuals with or without these markers did not decrease after several generations of selfing and backcrossing. There was a significant difference, 25.98 and 24.04 cN tex−1, 25.68 and 24.03 cN tex−1, and 26.14 and 23.66 cN tex−1, in the mean of fiber strength with and without SCAR4311920, UBC301933, and OPM071047, respectively. Additionally, the fiber strength means of homozygote plants with and without SSR1521130 marker were 25.94 cN tex−1 and 24.03 cN tex−1. Their difference was significant too. The mean fiber strength of heterozygote identified by SSR marker SSR1521130 was 24.94 cN tex−1. The difference between the heterozygote and other genotypes was not significant (Shen et al., 2001). It was concluded that MAS done to increase fiber strength is only possible with this major QTL, but a SCAR marker that is highly reproducible and economical makes it possible to screen

Table 2. Performance of fiber strength with and without DNA markers in (SM 3 x 7235) BC1F2 plants in 2000.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Fiber strength cN/tex</th>
<th>UBC301933</th>
<th>OPM071047</th>
<th>SCAR4311920</th>
<th>SSR1521130</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plants amplified</td>
<td></td>
<td>129</td>
<td>35</td>
<td>110</td>
<td>54</td>
</tr>
<tr>
<td>Fiber strength cN/tex</td>
<td>25.68</td>
<td>24.03</td>
<td>26.14</td>
<td>23.66</td>
<td>25.98</td>
</tr>
<tr>
<td>Difference</td>
<td>1.65</td>
<td>2.48</td>
<td>1.94</td>
<td>0.91</td>
<td>1.00</td>
</tr>
<tr>
<td>Variation</td>
<td>7.74</td>
<td>2.48</td>
<td>6.66</td>
<td>3.56</td>
<td>6.86</td>
</tr>
<tr>
<td>t Test</td>
<td>4.48</td>
<td>6.97</td>
<td>1.78</td>
<td>3.56</td>
<td>5.36</td>
</tr>
</tbody>
</table>

† MPT means marked plant type and WMPT without marked plant type.
‡ HH: homozygous for SSR marker, Hh heterozygous, and hh without SSR marker.
large breeding populations. Furthermore, a SCAR markers based on PCR technology facilitate molecular analysis of marker-assisted breeding selection.

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