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QTL mapping in A-genome diploid Asiatic cotton and their congruence analysis with AD-genome tetraploid cotton in genus *Gossypium*

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Abstract

Asiatic cotton (*Gossypium arboreum* L.) is an Old World cultivated cotton species. The sinense race was planted extensively in China. Due to the advances in spinning technology during the last century, the species was replaced by the New World allotetraploid cotton *G. hirsutum* L. *Gossypium arboreum* is still grown in India and Pakistan and also used as an elite in current cotton breeding programs. In addition, *G. arboreum* serves as a model for genomic research in *Gossypium*. In the present study, we generated an A-genome diploid cotton intraspecific genetic map including 264 SSR loci with three morphological markers mapped to 13 linkage groups. The map spans 2,508.71 cM with an average distance of 9.4 cM between adjacent loci. A population containing 176 $F_{2:3}$ families was used to perform quantitative trait loci (QTL) mapping for 17 phenotypes using Multiple QTL Model (MQM) of MapQTL ver 5.0. Overall, 108 QTLs were detected on 13 chromosomes. Thirty-one QTLs for yield and its components were detected in the F_2 population. Forty-one QTLs for yield and its components were detected in the $F_{2:3}$ families with a total of 43 QTLs for fiber qualities. Two QTLs for seed cotton weight/plant and lint index and three QTLs for seed index were consistently detected both in F_2 and $F_{2:3}$. Most QTLs for fiber qualities and yields were located at the same interval or neighboring intervals. These results indicated that the negative correlation between fiber qualities and yield traits may result from either pleiotropic effect of one gene or linkage effects of multiple closely linked genes.

Keywords: *Gossypium arboreum*; microsatellite; fiber quality; yield traits; QTL mapping

Introduction

Cotton (*Gossypium* spp.) is the world's most important source of natural fiber and edible oil. The genus *Gossypium* is comprised of 45 diploid and 5 allotetraploid species (Fryxell, 1992). Four species of *Gossypium* are presently cultivated, including two New World tetraploid species, *G. hirsutum* L. and *G. barbadense* L. (2n=4x=52) and two Old World diploid species, *G. arboreum* L. and *G. herbaceum* L. (2n=2x=26). Asiatic cotton, *G. arboreum*, was domesticated over 2,000 years ago in China following its introduction from India. This species has since been under cultivation (Xiang and Shen, 1989). *Gossypium arboreum* remains a germplasm resource in present cotton breeding programs due to numerous desirable traits for successful cotton production. Landrace genetic diversity in *G. arboreum* race *sinense* was assessed using microsatellite or simple sequence repeats (SSR) (Guo et al., 2006; Liu et al., 2006). Molecular data are valuable in examining the levels of genetic variation among *G. arboreum* accessions. These data can be used to develop mapping population to construct diploid cotton genetic linkage maps and subsequently tag economically important traits.

Progress in tetraploid cotton genome and QTL mapping and marker-assisted selection (MAS) has been extensively

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Abbreviations: Ht, plant height; FBN, fruit branch number/plant; PFBN, potent fruit branch number/plant; SCW, seed cotton weight/plant; LW, lint weight; BN, boll number/plant; BW, boll weight; SN, seed number /boll; LP, lint percent; SI, seed index; LI, lint index; FL, fiber length; FS, fiber strength; FM, fiber micronaire; FE, fiber elongation; FU, fiber uniformity ratio; SFI, short fiber index.

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investigated (Reinisch et al., 1994; Jiang et al., 1998; Shappley et al., 1998; Yu et al., 1998; Brubaker et al., 1999; Jiang et al., 2000a, 2000b; Ulloa et al., 2000, 2002; Zhang et al., 2002; Lacape et al., 2003; Lin et al., 2003; Zhang et al., 2003; Mei et al., 2004; Nguyen et al., 2004; Rong et al., 2004; Han et al., 2004; Lacape et al., 2005; Song et al., 2005; Desai et al., 2006; Rong et al., 2007). However, little work in the Asiatic cotton genome and QTL mapping has been accomplished due to low rate of polymorphism among A-genome *Gossypium* species (Brubaker et al., 1999; Rong et al., 2004). Recently, development of EST-SSR has resulted in the availability of a large number of SSR markers, which counteract the former constraints of modest levels of A-genome polymorphisms. An intraspecific Asiatic cotton genetic map was generated using 267 SSR loci from an F_2 population derived from a cross between *G. arboreum* cultivars (Ma et al., 2008). In this study, we used the linkage map to tag QTLs associated with fiber qualities, yield and its component traits. This study is helpful to better understand the evolutionary and genomic relationships of diploid and tetraploid cotton cultivars, which is vital to harness the extant genetic diversity for cotton breeding regimes.

Materials and methods

Plant materials and population generation

An F_2 population of 189 individuals was generated from an intraspecific hybrid cross between two Asian cultivated species, Jianglingzhongmian (JLZM) and Zhejiangxiaoshanlvshu (ZJXSLS) (AA, 2n=26) (Ma et al., 2008). The studies also demonstrated that both fiber- and yield-related traits between JLZM and ZJXSLS show significant difference at $P<0.01$ and $P<0.05$ (Table 1), which is the base for QTL mapping.

Cultivars JLZM and ZJXSLS were crossed in 2004. In winter, F_1 seeds were grown in Hainan Province, China to produce F_2 seeds. One hundred and eighty-nine F_2 were grown and phenotyped at the Jiangpu Breeding Station, Nanjing Agricultural University (NAU), and used to extract DNA in 2005. Subsequently, 176 $F_{2:3}$ families self-pollinated from individual F_2 selfed-pollination were planted with a randomized complete block design in two replications at NAU in 2006. Fifteen 30-day-old seedlings were transplanted to a one-row plot. Qualitative traits including stem hairs (*H*), anthocyanins (*R*), and recessive naked seeds (*n*) were scored as morphologic markers for each individual of F_2 and $F_{2:3}$ plants. Each individual plant of F_2 population and five plants in middle part of each row of $F_{2:3}$ population were tagged for scoring and harvesting cotton seeds. Yield and its component analysis included the following variables: plant height (Ht), fruit branch number/plant (FBN), potent fruit branch number/plant (PFBN), seed cotton weight/plant (SCW), lint weight/plant (LW), boll number/plant (BN), boll weight (BW), seeds number/boll (SN), lint percent (LP), seed index (SI), and lint index (LI). Fiber quality tests, including fiber length (FL), fiber strength (FS), fiber micronaire (FM), fiber elongation (FE), fiber uniformity ratio (FU), and short fiber index (SFI), were conducted at the Supervision, Inspection and Test Center of Cotton Quality, Ministry of Agriculture, China. Because the values of other fiber qualities were not available for some F_2 plants using the present fiber test machine when Micronaire value is over 8, QTL tagging for fiber-related traits was not conducted in F_2 population.

QTL mapping

Student's *t*-test was conducted for differences in 6 fiber qualities and 11 yield traits between JLZM and ZJXSLS. Trait means and correlations were calculated using SPSS13.0 software (SPSS, Chicago, Illinois, America). The intraspecific Asiatic cotton genetic map developed by Ma et al. (2008) was used to identify QTLs for fiber quailties, yield and its components using MapQTL 5.0 (van Ooijen, 2004). Analysis was conducted with a mapping step size of 2 cM and 10 maximum background marker loci in a stepwise regression at $P\leqslant 0.05$. *LOD* critical values for MQM analysis were determined using 1,000 random permutations. The position with the maximum *LOD* score on a linkage group was considered as the QTL position. The additive and dominance effects and the variance explained by each QTL were also obtained. The ± sign of each QTL 'additive effect' indicated an increase or reduction of the JLZM allele effect on the trait. Gene action was determined based on the dominance average as described by Stuber et al. (1987): additive (A), $|d/a| = 0–0.2$; partial dominance (PD), $|d/a| = 0.21–0.80$; dominance (D), $|d/a| = 0.81–1.20$; and overdominance (OD), $|d/a|>1.20$. QTL nomenclature was carried out as following Shen et al. (2005). The graphic presentation of the linkage maps and QTLs was created using MapChart2.1 (Voorrips, 2002).

Results

Phenotypic variation for fiber qualities yield and its components

The phenotypic data of fiber qualities and yield traits from the F_2, $F_{2:3}$ families and parents are summarized in Table 1. Student's *t*-test results indicated significant differences in six fiber qualities and 11 yield traits between JLZM and ZJXSLS, with the exception of fiber Micronaire and fiber uniformity ratio. To test the cotton genetic map utility, we analyzed 6 fiber-related and 11 yield and its