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AN INTEGRATED SSR-STS-SRAP-RAPD GENETIC MAP USING RECOMBINANT INBRED LINE POPULATION IN TETRAPLOID COTTON

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Abstract

An upland cotton germplasm, NM24016 derived from an interspecific cross between *Gossypium hirsutum* and *G. barbadense* was used to cross with Pima 3-79 to develop a recombinant inbred line (RIL) population. A total of 166 markers, consisting of SSRs, RAPDs, STSs, and SRAPs were developed for the mapping population. Twenty-eight linkage groups were constructed from 142 markers. Comparative marker mapping between F2 and RIL populations indicated a linkage map expansion in the RIL population, which strongly supports the strategy to develop the RIL population for more accurate genetic mapping. Other 24 markers that are polymorphic between the two parents did not segregate in the RIL population, indicating selective elimination on the related chromosomal regions.

Introduction

Upland cotton, *Gossypium hirsutum* (AD1) is a cultivated tetraploid species grown worldwide with high yield and wide adaptation, while its closest relative, *G. barbadense* (AD2) is grown in limited areas for its extra long, strong and fine fiber. During the most part of the past century, cotton breeding had made significant contributions to increase cotton yield, improve fiber quality and enhance biotic tolerance. Current and obsolete cultivars and strains in Upland cotton have been and still are the main sources in cotton breeding programs worldwide. The desirable and amenable genetic variations for breeders are limited or lacking or difficult to dissect. Due to the narrow genetic base of cotton germplasm that cotton breeders have been utilizing and low efficiency of traditional selection methods, varietal improvement in cotton has been slowed down in the past 10-15 years in the U.S. In fact, the past 10 years has seen cotton yield stagnant. Many factors have been discussed for the contributing causes: narrow germplasm base, shift in breeding method to backcrossing for transgenic introgression, nematodes and weather changes, among others.

Great genetic diversity and many desirable or potentially desirable genes or traits from *G. barbadense* have encouraged cotton geneticists working on interspecific hybridization for the past century. But the success is limited except for fiber quality improvement in Acalas. The modern DNA marker technology has renewed the interest in this area with many QTLs reported from several segregating populations between Upland cotton and Pima cotton, *G. barbadense*. However, genetic distance between the two species is so great in terms of DNA polymorphism, gene mutations, gene order and cryptic chromosomal structure differences that has created tremendous reproductive barrier, resulting in hybrid breakdown, hybrid sterility and gene selective elimination. That would cause inaccurate marker and gene mapping. To avoid the complication of the gene mapping, one can use some well introgressed lines with sufficient germplasm from Pima cotton in the Upland cotton background or vice versa. The intermediate homozygous type can serve as a bridge to cross with the two cultivated species for more precise marker mapping, gene tagging and introgression.

The objectives of the present study are, (1) to develop a recombinant inbred line (RIL) population from a cross between Pima 3-79, genetic standard for Pima cotton and NM 24016, an intermediate upland cotton line with tremendous germplasm introgression from Pima; and (2) to construct molecular maps using F2 and the RIL population. Our long term goals are, (1) to identify chromosomal segment for species differentiation and reproductive barriers by comparing the maps with that from another RIL population derived from TM-1 (Upland cotton genetic standard) x NM 24016; and (2) to identify quantitative trait loci (QTL) for the underlying genetic architecture of morphological and quantitative differences between the two species, of which some desirable QTLs will be utilized in our Acala cotton breeding program.

Materials and Methods

Mapping Population

NM 24016 was crossed as male to Pima 3-79 and the resulted F1 was grown in the greenhouse for selfing to generate F2 seed. The F2 and the following generations have been grown in the same greenhouse for generation advancement. Starting from F2, individuals were harvested separately to advance to the next generation using single seed descent (SSD).

DNA Preparation

One hundred seventy-six F2 plants and forty individual F7 plants were genotyped using morphological, SSR markers. Genomic DNAs were extracted from leaf tissues harvested in the greenhouse using the method of Zhang and Stewart (2000) or the DNeasy Plant Mini Kit (Qiagen, Santa Clarita, CA) following the manufacture's instructions. The DNA concentration was determined by a Fluorometer and then diluted to 10 ng/µl.

SSR Marker Analysis

The SSR primers were labeled with fluorescent dyes (Applied Biosystems Inc, Foster,CA). The PCR reactions were performed using a Perkin-Elmer 9700 Thermocycler in a 10-µL reaction solution containing 10 ng DNA template, 0.15 µM primers, 0.05 mM each dNTPs, 1X GeneAmp PCR Buffer, 2.5mM $MgCl_2$, and 0.4 units of AmpliTaq® Gold DNA polymerase. The PCR conditions were following: 12 min at 95°C, followed by 40 cycles of 15 s at 93°C for DNA denaturing, 30 s at 55°C for primer annealing and 1 min at 72°C for extension with a final extension for 7 min at 72°C. The finished PCR samples were stored at 4°C until use. The PCR products were then diluted 1:10 with sterile deionized water and separated by 12cm-long, 5% polyacrylamide gel at 750V for 1.5 hours on an ABI PRISM® 377 sequencer. The gel images were analyzed using the GeneScan software (Version 3.1.2, ABI) to size each amplified PCR bands.

SRAP Marker Analysis

The SRAP primers were fluorescent-labeled and screened on parental DNA, GB3-79 and NM24016. PCR condition was modified from Li and Quiros (2001). Polymorphic primers were tested on the F7 RIL population and the PCR products were separated on an ABI sequencer using similar protocol to SSR marker analysis. Rox 500 size standard and 36cm-long 5% polyacrylamide gel were used instead.

STS Marker and RAPD Marker Analysis

Standard PCR conditions were adopted to screen STS primers on the population. UBC RAPD primers were used in PCR profile as described in Zhang and Stewart (2000) for F7 population mapping. PCR products were separated on 1.4% agarose gel.

Linkage Map Construction

Mapmaker Macintosh V2.0 (1993, Du Pont Co. Wilmington, DE) was used to construct linkage groups using LOD 4.0 and recombination fraction 0.45. Female parent was GB 3-79 and coded as 'a'. Male parent was NM24016 and coded in Mapmaker as 'b'. Heterozygotes containing both parental co-dominant bands were coded as 'h'. Haldane mapping function was used to calculate the genetic distance between markers in centi- Morgan (cM).

Results and Analysis

Morphological Characterization of Parents

Morphologically, NM 24016 looks like an Upland cotton type (Table 1), but it possesses Pima cotton characteristics, such as sub-okra leaf shape, yellow pollen, and longer and stronger fiber than Upland cotton. DNA marker data showed that it shared 60% markers with Upland cotton and 40% markers with Pima cotton.

Construction of Molecular Map for F2 Population

Using LOD of 2.0 and recombination fraction of 0.40, the 73 SSR markers were constructed into 14 linkage groups containing 64 SSR markers by Mapmaker. Nine SSR markers were unlinked. One linkage group had 29 SSR markers which were distributed into four subgroups when the critical LOD was set at 3.0 and recombination fraction at 0.40. Nine linkage group/subgroups were assigned to specific chromosomes or arms based on the aneuploid analysis.

Construction of Molecular Map for F7 RIL Population

Out of a total of 166 markers, 149 SSR, 8 SRAP, 5 STS, and 4 RAPD markers were screened on the RIL population. Of 149 SSR markers, 51 had been used for F2 population. In the F7 RIL population 24 SSR markers did not show polymorphism even though they were polymorphic between the two parents. In most of these markers the RIL population possessed only maternal (3-79) genotype (Table 2). Interestingly, many of these markers are located on Chromosome A03 and D08. Some of them are located on homoeologous chromosomes. Using LOD of 4.0 and recombination fraction of 0.45, the other 142 markers were constructed into 28 linkage groups containing 100 markers by Mapmaker.

Comparison of F2 and F7 RIL Mapping

Eight linkage groups involving 37markers from the F7 RIL population were consistent with the linkage map based on the F2 marker data. (Figure 1) Genetic distances between common linked markers on the five linkage groups for the two mapping populations showed similar results. Other small linkage groups also showed consistent results with the published maps (maps not shown) (Lacape et al., 2003) However, discrepancies between our linkage maps and the published ones do exist, warranting further investigations.

References

Li, G. and C.F.Quiros. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor Appl Genet. 103:445-461.

Lacape, J.M, T.-B. Nguyen, R.G. Cantrell, B. Burr, and B. Hau. 2003. A combined RFLP-SSR-AFLP map of Tetraploid cotton based on a *Gossypium hirsutum* x *Gossypium barbadense* backcross population. Genome 46:612-626.

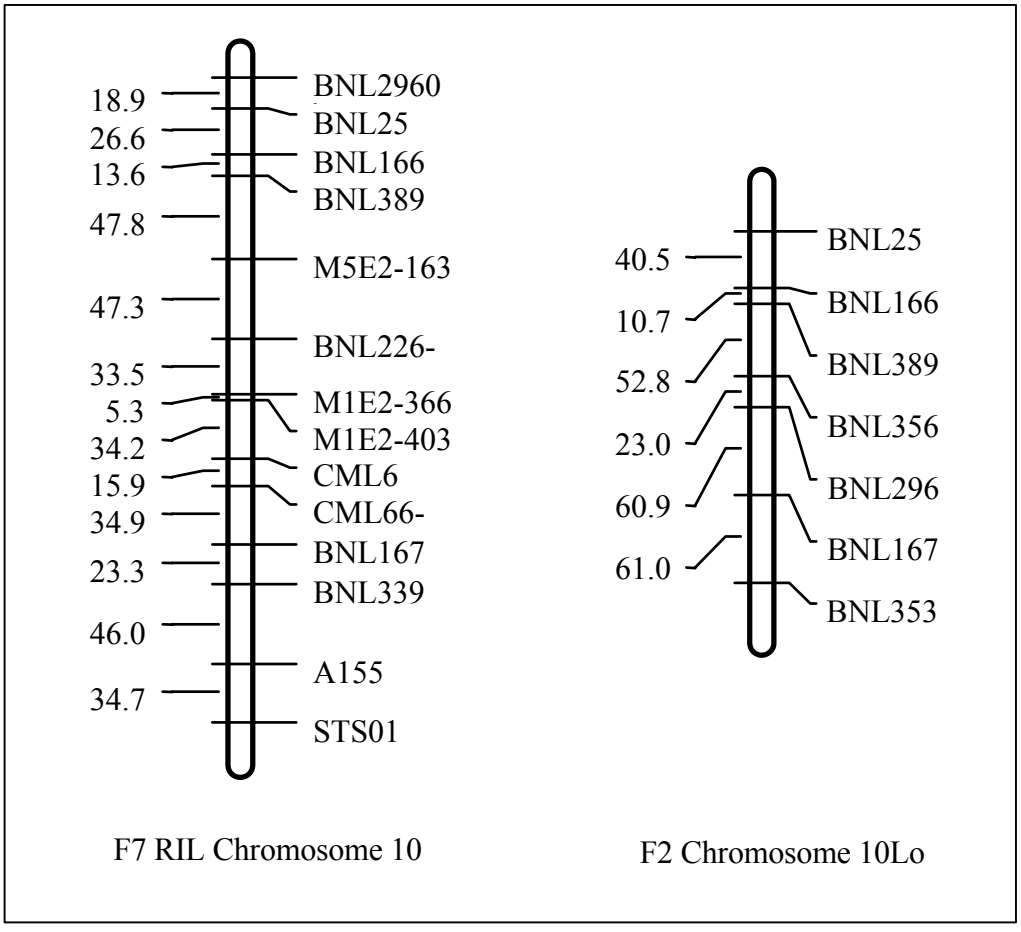
Zhang J.F. and J. McD. Stewart. 2000. Economical and rapid method for extracting cotton genomic DNA. J. Cotton Sci. 4:193-201.

Table 1. Comparison of the two parents, NM24016 and Pima 3-79

Trait	Pima 3-79	NM 24016	Genetics
Leaf shape	Sub-okra	Sub-okra	One gene
Leaf size	Large	Medium	Polygene
Leaf color	Light green	Green	Polygene
Plant height	Tall	Medium	Polygene
Flower color	Yellow	Cream	One gene
Pollen color	Yellow	Yellow	One gene
Petal spot	Presence	Absence	One gene
Seed fuzz	Absence	Presence	One gene
Fiber yield	Low	Relatively low	Polygene
Boll size	Small	Medium	Polygene
Fiber length	Extra long	Long	Polygene
Fiber strength	Very Strong	Strong	Polygene
Fiber fineness	Very good	Good	Polygene

Table 2. SSR markers identified to be presented as identical phenotype in F7 RIL populations

SSR Marker	Chr. Posit.	Genotype from	SSR Marker	Chr. Posit.	Genotype from
BNL3408-2	c3sh	NM24016	BNL2440-1	c15	GB3-79
BNL3029-1	c5sup	NM24016/GB3-79	BNL1611	D08	GB3-79
BNL1440-1	c6sh	GB3-79	BNL1690	D08	GB3-79
BNL1317-2	c9	GB3-79	BNL3029-2	D08	GB3-79
BNL3280-1	c18sh	GB3-79	BNL852	D08	GB3-79
BNL2961	A02/D03	GB3-79	BNL1414-1	c23	GB3-79
BNL1034-2	A03	GB3-79	BNL3511-2	c23	GB3-79
BNL1681-1	A03	GB3-79	BNL3482-3	c26lo	NM24016
BNL3411	A03	GB3-79	BNL1438	A01	GB3-79
BNL4094	A03	GB3-79	BNL1521	D03	GB3-79
BNL836-2	A03	GB3-79	BNL3860-2	D03	GB3-79
BNL2597-2	na	GB3-79	BNL3368-2	na	GB3-79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A155
STS01
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10.7
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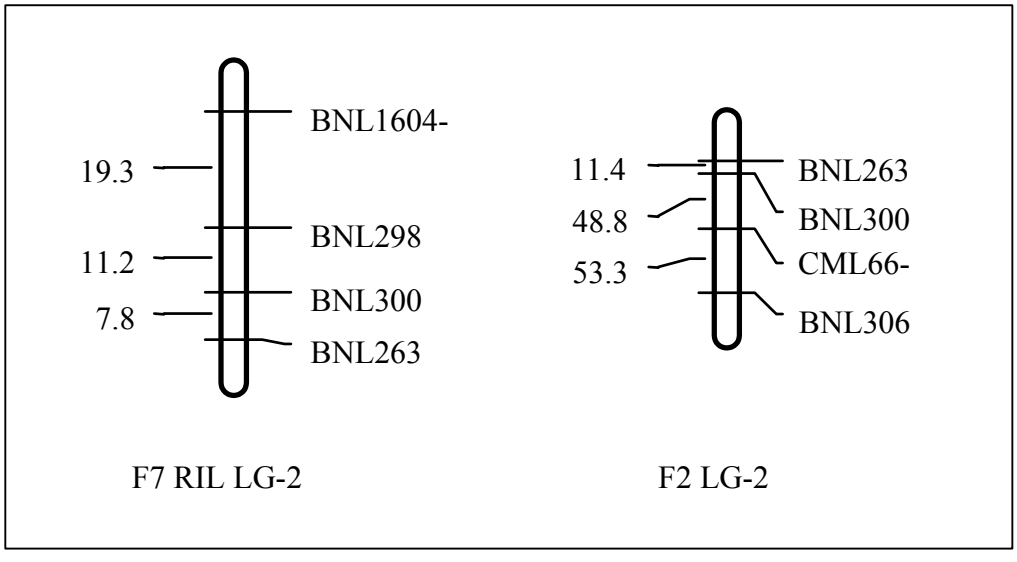
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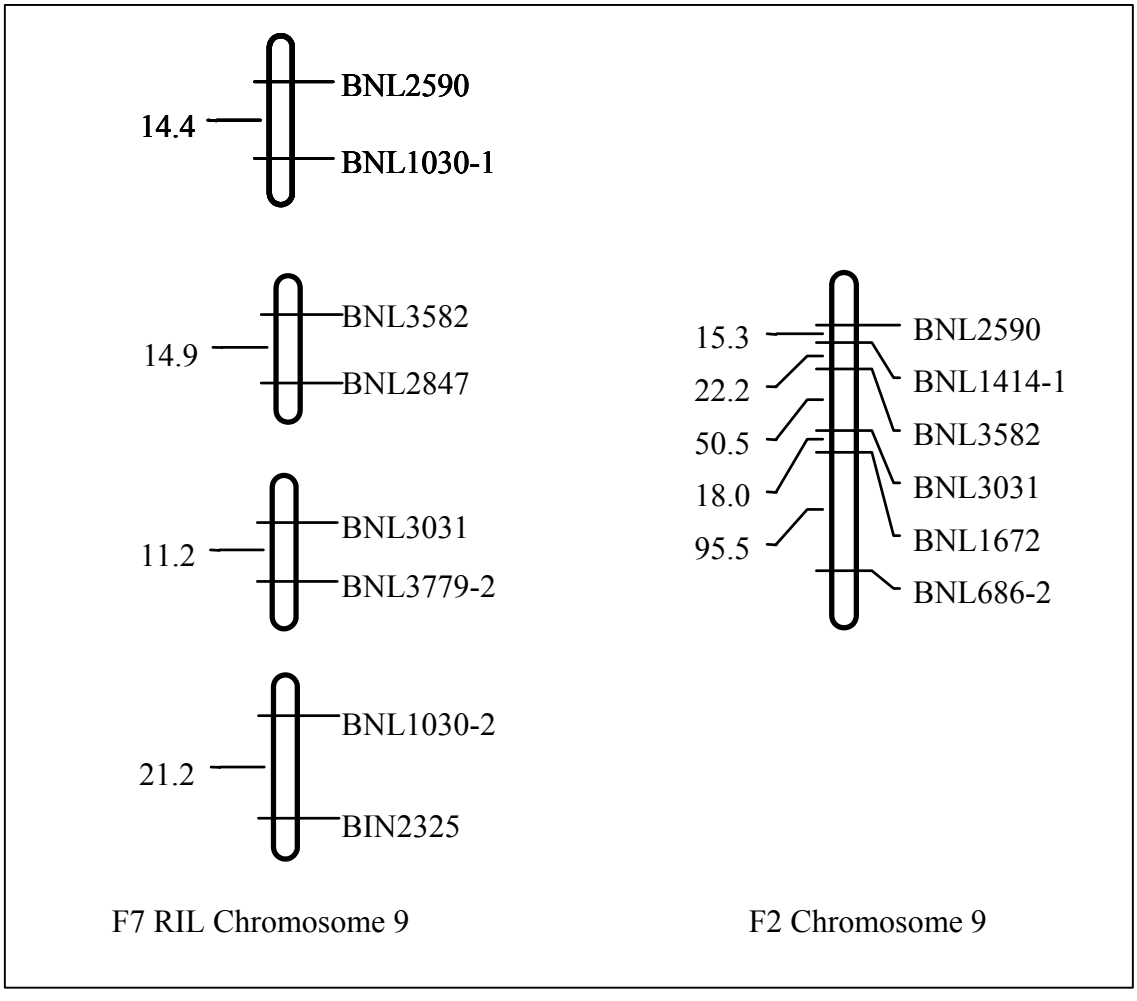
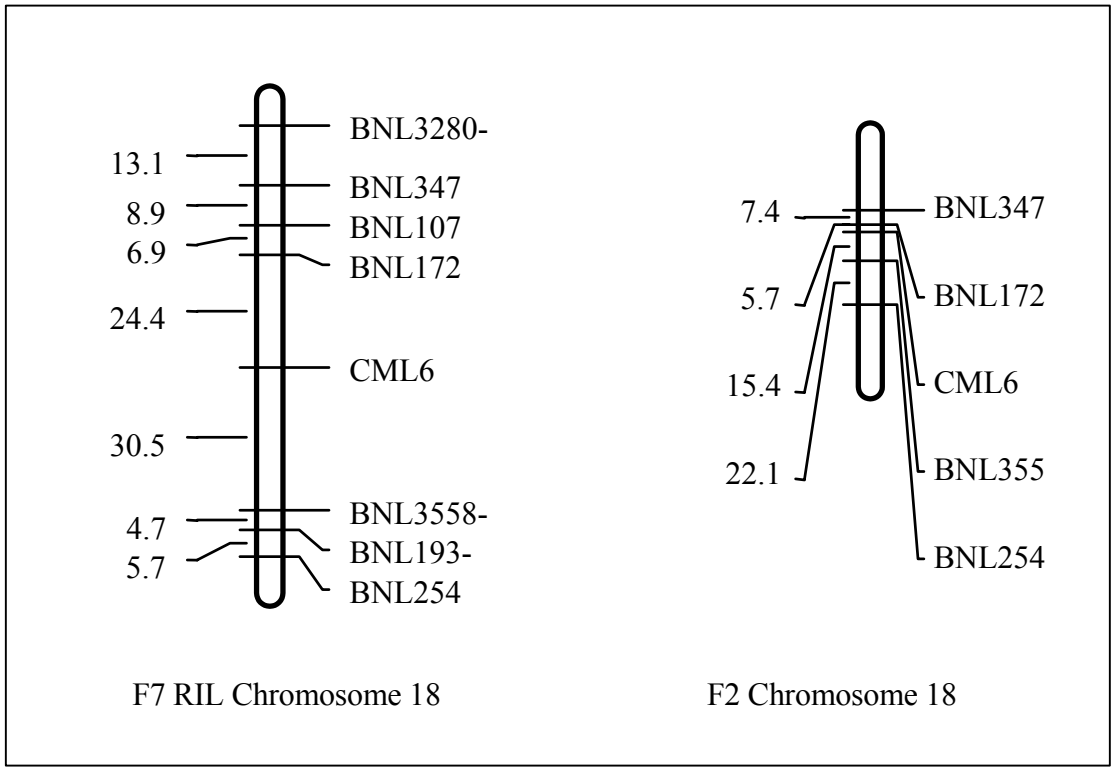


F7 RIL Chromosome 9

F2 Chromosome 9



F7 RIL Chromosome 18

F2 Chromosome 18

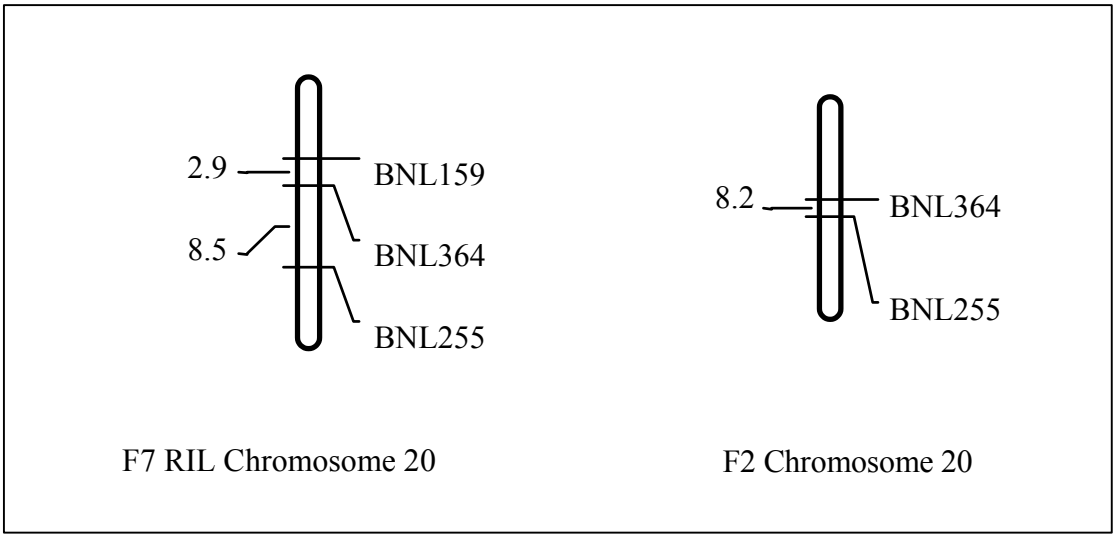


Figure 1. Comparative linkage maps in NM24016 x Pima 3-79 F7 RIL and F2 population. a. Genetic distance (cM); b. Marker Id.