An RFLP and QTL Linkage Map in *Gossypium hirsutum* L.

J.N. Jenkins, Z. Shappley, and J.C. McCarty, Jr.
United States Department of Agriculture: Agricultural Research Service
Box 5367, Mississippi State, MS 39762; and
Mississippi State University, Mississippi State, MS 39762

ABSTRACT

Ninety-six $F_2.F_3$ bulk sampled families of Upland Cotton, Gossypium hirsutum L. from a cross of MARCABUCAG8US1-88 X HS 46 were analysed with 129 probe/enzyme combinations resulting in 138 RFLP loci. There were 84 co-dominant markers of which 76 fit a normal 1:2:1 ratio. There were 54 dominant markers of which 50 fit a normal 3:1 ratio. Using MAPMAKER\EXP program these were arranged into 31 linkage groups with 120 linked loci and 18 unlinked loci. These covered 865 cM or 18.6% of the estimated cotton genome. We used the mixed model analysis of Zhu and Weir (1998) to analyse QTLs associated with 19 agronomic and fiber traits scored mostly in $F_2.F_5$ families. The model also provided estimates of the additive and dominance genetic effects. We mapped 100 QTLs to 60 maximum likelihood locations in 24 linkage groups. Several QTLs influenced more than one trait. For example, in linkage group 14 we found a QTL with significant genetic effects for micronaire, and three Arealometer measurements. In linkage group 19 four closely linked QTLs had significant effects on strength, fineness, and maturity of fiber.

Introduction

Meredith (1992) reported the first RFLP evaluations in cotton when he studied heterosis and varietal origins of several cultivars using RFLP probes. The only RFLP linkage maps in a cross of two upland cotton (*G. hirsutum)* lines have been reported by Shappley (1994, 1996), Shappley *et al.* (1996), Shappley *et al.* (1998). Molecular markers provide increased potential for gathering useful information for cotton improvement. The identification of QTLs controlling traits of interest to breeders of upland cotton and their association with RFLP markers was the focus of our research.

Material and Methods

The two cultivars chosen for our study are very diverse in pedigree and in RFLP patterns. Agronomic and fiber properties are also very diverse between these two parents. We crossed MARCABUCAG8US-1-88 X HS 46 and screened 9 F_1 plants for RFLP variability. Some variability was found so we chose one F_1 plant and self pollinated it for the beginning of segregating generations for this study. The RFLP analysis was conducted by the commercial laboratory, Biogenetic Services, Brookings, SD. This laboratory developed most of the probes from a cDNA library using leaf material collected from six diverse cultivars. We grew $F_2.F_3$ families and harvested leaf samples. Shappley *et al.* (1998) contains details of the RFLP analysis All RFLP probes were scored visually on x-ray film after exposure to the proper conditions. Parameters for detection of linkage groups of RFLP loci were a LOD score of 3.0 or greater and a genetic distance of 50cM. The fiber analysis was by Starlab Inc., a commercial fiber laboratory, in Knoxville, TN. Lint for fiber analysis was from hand harvested bolls on plants in $F_2.F_5$ families, ginned on a 10-saw gin.

Results and Discussion

Most RFLP loci segregated normally for co-dominant or dominant loci. Twelve of 138 loci showed distorted segregation. Parallel research with cytogenetic deficiency stocks showed that three of the 12 markers also segregated abnormally in the cytogenetic studies. The cause of abnormal segregation is not known but abnormal segregation is not unusual with molecular markers. Schon *et al.* (1993) reported an excess of heterozygotes in his study of corn. Saha (1989) reported an excess of heterozygotes in his analysis of isozyme alleles in cotton. The detailed genetic linkage map is reported in Shappley *et al.* (1998).

We found 31 linkage groups with 2 to 10 markers each in our cross of two *G. hirsutum* lines. Reinisch *et al.* (1994) developed a detailed RFLP map of cotton; however, they used an interspecifc cross of *G. hirsutum* L. race "palmeri" by *G. barbadense* L. accession K101. Jiang *et al.* (1998) published a QTL linkage map showing several QTLs for fiber traits in an interspecific cross. Breeders have known for a long time that interspecific crosses show abnormal segregation. Thus, these linkage map developed from an intraspecific *G. hirsutum* cross may be more useful to breeders.

Segregation of phenotypic traits in our research, except number of nodes, was normal based upon the skewness and kurtosis values suggesting that these data were suitable for QTL analysis. The phenotypic mean and range for each trait are shown in Table 1. These traits are of interest to cotton breeders and thus the QTLs

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reported should be useful. We found QTLs associated with each trait except fiber perimeter. The number of QTLs associated with each trait ranged from two to 18, Table 2. Micronaire and elongation had the greatest number of QTLs. We used a likelihood ratio of greater than 6.63 (significant at the 0.01 level of probability) for acceptance of a significant QTL. Most likelihood ratios were significant at the 0.005 or 0.001 levels of probability, providing strong evidence that the QTL was associated with the marker and the trait of interest. Fiber traits measured in the F_5 generation made on 25 individual plants in each family provide an exceptional measure of fiber properties.

We found 100 QTLs that mapped to 60 maximum likelihood locations in 24 linkage groups. As expected, the most frequent association of traits with a common QTL location was for fiber traits affecting fineness and maturity of fiber. The number of QTLs, number of linkage groups with a QTL, and the largest additive or dominance genetic effect of QTL alleles for each trait are shown in Table 2.

Examples of QTLs and genetic effects for two linkage groups (14 and 19) are shown in Table 3. Considering all linkage groups, four QTLs for fiber strength in four linkage groups gave dominance genetic effects of more than 1 gram per tex. Several QTLs were detected with genetic effects greater than one micronaire unit. For example, in linkage group 14, one QTL near marker F4B4RV and another QTL near markers C117C5RV, C117C5RI and F2E6RI significantly affect fineness and maturity traits.

Shappley (1996) provided the first linkage map of QTLs in a cross of upland cottons. However, while carefully examining these data in preparation for writing a manuscript and preparing for this conference, we discovered a computer coding error in the QTL data of Shappley (1996). Thus, the QTL map data in Shappley (1996) are not correct.

The putative location of these QTLs were established with a high likelihood ratio. However, the values given for these RFLP loci and QTLs do not necessarily provide the physical map distance. A physical linkage map needs to be developed as well as more refined molecular marker linkage maps. Physical maps of chromosomes should be very helpful when attempting to clone QTL genes. Research is under way in our laboratory to assign these linkage groups to chromosomes. See the paper by Saha *et al.* (1998) at this conference.

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Table 1. Phenotypic data of agronomic and fiber traits for the families derived from a cross of MARCABUCAG8US-1-88 as parent 1 X HS 46 as parent 2.

Trait	Mean	SD	Maximum	Minimum	Skewness	Kurtosis
Sdx	11.13	1.00	13.50	8.20	-0.10	-0.17
Lp	35.70	1.30	39.38	33.06	0.33	-0.20
Mic	4.24	0.36	4.95	3.29	-0.45	-0.08
E1	6.72	0.48	7.79	5.57	-0.34	-0.25
T1	20.74	1.00	23.11	18.04	0.20	-0.12
S150	0.55	0.02	0.59	0.51	-0.04	-0.70
S12.5	1.12	0.03	1.19	1.04	-0.07	-0.40
Ah	497.72	36.15	601.81	424.44	0.48	0.02
A	470.88	30.79	556.02	409.06	0.49	-0.02
Im	1.68	0.12	2.04	1.43	0.28	-0.02
Mat	86.01	4.67	95.46	72.06	-0.30	0.02
Per	44.79	2.74	49.43	40.15	0.02	-0.42
Wtfn	3.73	0.28	4.34	3.06	-0.20	-0.17
Wall	2.68	0.22	3.25	2.14	0.06	-0.08
Nodes	18.35	1.26	22.27	14.63	0.11	1.59
Nfb	6.91	0.36	7.88	6.19	0.59	0.25
Hgt	77.76	6.02	92.08	64.94	-0.09	-0.50
Hnrat	4.28	0.39	5.35	3.43	0.14	-0.05
B1	48.86	17.14	92.11	11.67	-0.07	-0.03

Table 2. Agronomic and fiber traits, number of QTLs, number of linkage groups with a QTL for the trait, and the largest additive and dominance effects.

Trait	No. of QTLs	No. Linkage Groups (1)	Largest Genetic Effect of a QTL Alleles	
			Additive	Dominance
Seed index	4	3	-2.00*	-0.91
Lint percent	5	5	1.95*	1.08*
Micronaire	15	13	-1.07**	0.81**
Elongation	18	18	-1.63**	-3.43**
T1 strength	6	6	0.66*	1.61*
SL 50%	2	2	-0.01*	-0.01*
SL 2.5%	5	5	-0.02*	-0.02*
Fiber Ah	6	5	-95.88**	-58.86*
Fiber A	6	5	-55.73**	-52.86*
Fiber Immaturity	4	4	-0.04*	-0.17*
Fiber Maturity	4	4	1.56**	6.50*
Fiber Perimeter	0	0		
Fiber wt. Fineness	7	6	0.73**	0.57**
Fiber wall thickness	8	7	0.57**	0.44**
No. of Nodes	3	3	-0.22	-0.92**
Node first fruiting branch	1	1	-0.01	0.27**
Plant height	2	2	-2.44**	-3.56*
Height to Node Ratio	2	2	-0.07	-0.37*
Bloom Rate	2	1	8.88	17.09*

(1) The number of different linkage groups with a QTL for this trait

*, ** significant at the 0.05 and 0.01 level respectively

Table 3. Selected examples of QTLs and genetic effects in linkage groups 14 and 19 which affect traits of interest for breeding upland cotton.

QTL Trait	Linkage Group	Maximum Likelihood Location	Likelihood Ratio	Estimate of Genetic Effects			
				Additive	± SE	Dominance	± SE
Mic	14	2.5	16.00****	1.07**	±0.30	0.54*	±0.22
El	14	2.5	16.93****	-1.63**	±0.41	-0.93**	±0.30
Ah	14	2.5	13.84****	-95.88**	±30.16	-45.55	±21.71
Wall	14	2.5	12.05****	0.57**	±0.18	0.29*	±0.13
A	14	4.5	13.34****	-55.77**	±18.75	-20.80	±14.92
Sdx	14	38.5	12.03***	-0.28*	±0.13	0.46	±0.25
A	14	42.5	16.77****	-9.92*	±4.14	17.02*	±8.41
Im	14	42.5	12.68****	-0.04*	±0.02	0.05	±0.03
Sdx	14	54.5	9.74***	-0.26*	±0.12	0.38*	±0.18
Mic	14	54.5	19.75****	0.11*	±0.04	-0.22**	±0.07
Ah	14	54.5	18.63****	-10.66*	±4.32	21.58**	±6.54
Mat	14	54.5	13.82****	1.56**	±0.57	-1.98*	±0.87
Wtfn	14	54.5	13.39****	0.04	±0.03	-0.17**	±0.05
Wall	14	54.5	20.64****	0.08**	±0.03	-0.13**	±0.04
El	19	48.5	11.61****	-0.48**	±0.18	-1.11**	±0.35
Mic	19	50.5	7.49**	0.33*	±0.14	0.69**	±0.26
A	19	50.5	6.66**	-23.16*	±11.65	-52.86*	±22.13
Ah	19	52.5	7.03**	-25.27	±13.51	-58.86*	±24.91
Wall	19	52.5	8.20***	0.15	±0.08	0.37*	±0.15
Im	19	54.5	8.65***	-0.06	±0.04	-0.17*	±0.08
Mat	19	54.5	8.63***	2.32	±1.68	6.50*	±3.02
T1	19	56.5	6.97**	0.66	±0.33	1.43*	±0.58

*, ** Significant at the 0.05 and 0.01 levels, respectively.

***, ****Significant at the 0.005 and 0.001 levels, respectively.