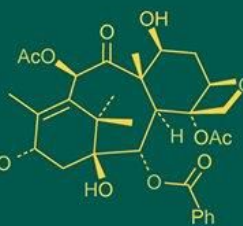
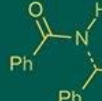
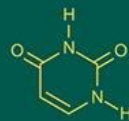
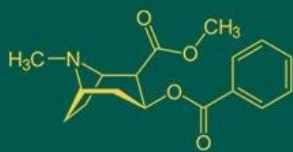


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Santoshkumar Magadam

1) Department of Cotton,
Centre for Plant Breeding and
Genetics, (TNAU),
Coimbatore, Tamil Nadu,
India

3) CSB–Regional Sericultural
Research Station, Central Silk
Board, Miran Sahib, Jammu,
India

NM Boopathi

Department of Plant Molecular
Biology and Bioinformatics,
Centre for Plant Molecular
Biology, TNAU, Coimbatore,
Tamil Nadu, India

R Ravikesavan

Department of Cotton, Centre
for Plant Breeding and
Genetics, TNAU, Coimbatore,
Tamil Nadu, India

Corresponding Author:

Santoshkumar Magadam

1) Department of Cotton,
Centre for Plant Breeding and
Genetics, (TNAU),
Coimbatore, Tamil Nadu,
India

3) CSB–Regional Sericultural
Research Station, Central Silk
Board, Miran Sahib, Jammu,
India

Identification of major QTLs for fiber quality traits in cotton using interspecific backcross mapping population

Santoshkumar Magadam, NM Boopathi and R Ravikesavan

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Abstract

A segregating interspecific backcross population BC₁F₁, (KC 3 x Suvin) x KC 3, was generated by crossing two diverse parents *Gossypium hirsutum* L. var. KC 3 and *Gossypium barbadense* L. var. Suvin. A genetic linkage map was constructed using 141 polymorphic SSR markers, which had 57 marker loci distributed over 19 linkage groups, assigned to 16 chromosomes and spanned 911.6 cM. Though, several QTLs were identified, two QTLs linked to fiber quality traits were found to be particularly important since they were major QTLs. A QTL region flanked by CIR150 and CIR407 on chromosome 25 was identified for micronaire with 14.7 per cent of phenotypic variation and the same region was reported elsewhere for micronaire. Another QTL region flanked by CIR219 and NAU3935 on chromosome 19 was identified for 2.5 per cent span length with 17.7 per cent of phenotypic variation. Hence, these two QTLs may play a potential role in future cotton breeding programme.

Keywords: Cotton, major QTL, SSRs, linkage map, fibre quality traits

Introduction

Cotton is an important natural fibre of global importance and generates high employment as it moves from cultivating field to textile mills to consumer. This crop has significant contributions in Indian economy by earning > 30 per cent of foreign exchange. Internationally, India ranks first in cotton area but occupies second position in production, next to China. Because of competition with synthetic fibres, textile manufacturers have adopted more efficient production machinery capable of generating more products per unit of time. Thus, the widespread use of high speed spinning technology has increased the demand for raw cotton fibre with higher strength. Besides fibre strength, length (2.5 per cent span length) and fineness (micronaire) are the other primary qualities that influence textile processing (Kohel, 1999) ^[1]. Thus, a long term challenge faced by cotton breeders is the simultaneous improvement of yield and fibre quality. Although conventional breeding methods have contributed much to the development of cotton cultivars, simultaneous improvement of fibre quality and yield is still difficult (Lin *et al.*, 2005) ^[2]. Often, these attempts had resulted in poor agronomic qualities of the progeny, distorted segregation, sterility, motes formation and limited recombination (Reinisch *et al.*, 1994) ^[3].

Recent developments in quantitative trait loci (QTL) mapping and marker assisted selection (MAS) offers new avenues to overcome the above said limitations. The identification of the stable QTLs affecting fibre traits across the genetic backgrounds would be more useful for efficient MAS. The development of DNA markers linked to the fibre quality QTLs would allow cotton breeders to trace this very important trait in early plant growing stages or early segregating generations (Boopathi *et al.*, 2011) ^[4]. Among the DNA markers, advent of simple sequence repeat (SSR) or microsatellite markers have brought a new, user friendly and highly polymorphic class of genetic markers for cotton. SSRs are simple, usually co-dominant, well dispersed throughout the genome and easily shared between labs *via* flanking primer sequence. Reddy *et al.* (2001) ^[5] suggested that the total pool of SSRs present in the cotton genome is sufficiently abundant to satisfy the requirements of extensive genome mapping (~5000 cM), QTL analysis and MAS for the development of highly productive *G. hirsutum* cultivars

with superior fibre quality and can facilitate efficient comparative QTL mapping.

The first and foremost requirement in QTL analysis is development of mapping population to study the segregation of molecular markers and trait of interest. Permanent mapping populations such as recombinant inbred lines (RILs) permit multi-location experiments and thus they are expected to be superior over F_2 population with regard to the power of QTLs detection. But those QTLs with large over-dominant effects would not be detected in RIL population because RIL express only additive effects. Further, genetics of F_2 progenies reveal all the allelic combinations and inter and intra genic interactions. In addition, development of RILs is a cost involving and time consuming process (Lacape *et al.*, 2005) ^[6]. Consequently, F_2 and backcross (BC) progenies are the most preferred populations since they are easy to construct, require a short time for development and can be constructed with minimum efforts as compared to other populations (Reiter *et al.*, 1992) ^[7].

The upland cotton (*G. hirsutum*) is the most extensively grown among the four cultivated *Gossypium* species. However, as revealed by all means of assessment, the level of genetic diversity is low in *G. hirsutum* especially among agriculturally elite types (Gutierrez *et al.*, 2002) ^[8]. Existence of low polymorphism in the commonly cultivated and locally well adapted cultivars hinders the linkage map construction and QTL analysis of fibre quality traits (Paterson *et al.*, 2003) ^[9]. Hence, use of interspecific cotton population offers significant level of polymorphism for efficient QTL mapping despite the fact that regional breeding program involves locally adapted intraspecific crosses. Identification of molecular markers linked to QTLs for fibre quality traits have already been reported using interspecific populations of *G. hirsutum* and *G. barbadense* (Lacape *et al.*, 2005 ^[6], Paterson *et al.*, 2003 ^[9], Lacape *et al.*, 2003 ^[10], Collard *et al.*, 2005 ^[11], Zhang and Stewart, 2000 ^[12], Iwata and Ninomiya, 2006 ^[13]).

Ideally, QTL mapping studies should be independently confirmed due to several factors that influence the QTL detection and validated in the local elite lines (Collard *et al.*, 2005) ^[11]. Furthermore, several studies have proposed that QTL positions and effects should be evaluated in independent populations, because QTL mapping based on typical population sizes results in a low power of QTL detection and a large bias of QTL effects (Collard *et al.*, 2005) ^[11]. Unfortunately, due to constraints such as lack of research funding and time and possibly a lack of understanding of the need to confirm results, QTL mapping studies are rarely confirmed. Hence, the objective of this study was to develop interspecific BC_1F_1 population derived from locally adapted *G. hirsutum* var. KC3 and *G. barbadense* var. Suvin for linkage map construction and QTL analysis of fibre quality traits using simple and anchored SSR markers.

Materials and Methods

Plant materials

Short duration upland cotton variety, *G. hirsutum* L. var. KC3, possessing short staple length but well adapted to rainfed tracts of Tamil Nadu, India was used as recurrent parent. A contrasting long duration *G. barbadense* L. var. Suvin, possessing exceptionally long and strong fibres and susceptible to sucking pests and drought, was used as donor parent. The two parents and 62 BC_1F_1 individuals were

grown in a field by following regular agronomic practices at Cotton Breeding Station, Tamil Nadu Agricultural University (TNAU), Coimbatore, India for evaluation of fiber quality traits and for providing leaf samples for linkage map construction.

Phenotyping

Observations on five important fibre quality traits were recorded on every replicated progenies of BC_1F_1 population. Matured bolls were collected from each plant, ginned and the lint was used to assess the fibre quality traits using high-volume instrument (HVI900 classic). The tested fibre quality traits included 2.5 per cent span length (mm), bundle strength (g/tex), fibre fineness (micronaire), uniformity ratio and elongation per cent. From these data, broad sense heritability for all the fibre quality traits was calculated besides regular statistical analysis.

Genotyping and Segregation analysis

Genomic DNA was isolated from the fresh leaves of parents and the 62 BC_1F_1 individuals and genotyping with SSR markers were done with established procedure (Zhang and Stewart, 2000) ^[12] and PCR products were separated on a 4% metaphor agarose gel. A total of 391 pair of SSR primer sequences that span all over the cotton genome were obtained from cotton marker database (CMD) (<http://www.cottonmarker.org/Downloads>). A sum of 141 polymorphic SSRs was used for genotyping the BC_1F_1 mapping population. The segregation pattern of SSR markers for all the BC_1F_1 individuals were scored as co-dominant fragments and this genotyping data was used for linkage map construction and QTL analysis for fibre quality traits. Initially, genotyping data of all the SSRs were used for Chi-square (χ^2) test using AntMap Ver. 1.2 for deviations from the expected Mendelian ratios with $P < 0.05$ (Iwata and Ninomiya, 2006) ^[13]. The SSRs, which have shown 1:1 segregation ratio for BC_1F_1 progenies were used for linkage map construction.

Linkage map construction and QTL analysis

Preliminary linkage analysis was performed using both AntMap Ver. 1.2 (Iwata and Ninomiya, 2006) ^[13] and MAPMAKER/EXP 3.0 (Lander *et al.*, 1987) ^[14]. Linkage groups were assigned to corresponding chromosomes using anchored SSR markers as per CMD. QTL analysis was performed using Windows QTL Cartographer V2.5 (Basten *et al.*, 1999) ^[15] by employing single marker analysis (SMA), interval mapping (IM) and composite interval mapping (CIM) methods. As per the results of 1000 permutation tests, a LOD threshold of ≥ 2.5 was set to identify the putative QTL linked to fibre quality traits.

Results

Genetic Variation and Heritability

Segregation data for all fibre quality traits in BC_1F_1 progenies was tested for genetic variation and heritability. The two parental lines differed significantly for all the fiber quality traits (Table 1). Transgressive segregation or continuous variation for all the investigated fibre quality traits showed the existence of wide genetic variation among the BC_1F_1 population and represented both the extremes of parental values for all the investigated traits. The heritability estimates ranged from 54.10 to 72.30 per cent in BC_1F_1 progenies (Table 1).

Linkage analysis and map construction

A total of 391 SSRs were used to screen for polymorphism between the parents KC3 and Suvin. Though, 162 SSRs showed polymorphism between parents, only 141 (41.43%) SSRs yielded unambiguous, scorable polymorphic loci and thus, they were chosen to genotype the BC₁F₁ population. Genotypic data on all the 141 polymorphic loci were utilized to construct linkage map using AntMap Ver. 1.2. All the SSRs were assigned and arranged with their respective chromosome number as per CMD. Linkage analysis was carried out individually for all the 26 chromosomes of cotton and genetic map was constructed (Figure 1).

The linkage map constructed using BC₁F₁ population, among the 141 SSR loci, 19 loci deviated from an expected 1:1 segregation ratio ($P \leq 0.05$). Thirteen per cent of the markers showed distorted segregation, indicating that SSRs are likely to segregate normally, although the population is not large. Among the distorted loci, 9 loci skewed towards the 'KC3' parent and 10 loci skewed towards the heterozygote. The markers that are segregating normally were analyzed further and it was noticed that 57 loci were found to be linked and the 84 loci were remained unlinked at LOD of 2.5. A total of 19 linkage groups were formed which were assigned to 16 chromosomes of cotton. The 19 linkage groups contained two to seven markers with an average length of 47.98 cM. The map covered a total of 911.6 cM and the average distance between adjacent markers was 16 cM. Maximum interval length of 44.9 cM was observed between CIR185 and BNL3995 on chromosome 5 and a minimum inter marker distance of 5.1 cM has been noticed between CIR039 and CIR143 on chromosome 26 (Figure 1).

QTLs associated with fibre quality traits

QTL analysis was carried out with QTL Cartographer V.2.5 by performing SMA, IM and CIM to find out the QTLs linked to fibre quality traits in cotton. Interesting outcome of this study was identification of two major QTLs by SMA, IM and CIM. The marker interval NAU3935 and CIR219, on chromosome 19 has been found to be associated with 2.5 per cent span length and explained 17.7% of phenotypic variation. Another QTL has been found to be associated with micronaire which was located on chromosome 25 with the marker interval CIR150 and CIR407 and contributed 14.7 per cent of phenotypic variation in BC₁F₁ progenies (Table 2).

Discussion

Heritability estimates are normally more helpful in predicting the gain under selection. In the present study, high heritability was observed for all the fiber quality traits which revealed that these traits were polygenic in nature and amenable for QTL analysis (Paterson *et al.*, 2003) [9]. However, the estimates of heritability are subject to environmental conditions and therefore may be used with great care and caution.

The present investigation was attempted to utilize BC₁F₁ progenies for detecting the genomic regions associated with fibre quality traits. Though, similar kind of linkage maps using the same type of mapping populations derived from interspecific crosses of *G. hirsutum* L. and *G. barbadense* L. have already been reported, it is important to validate the identified QTLs in the locally adapted accessions for

effective MAS (Boopathi *et al.*, 2011) [4]. A population size of 62 BC₁F₁ progenies were used for linkage map construction and QTL analysis in the present study. The earlier studies by Lacape *et al.* (2003) [10], Nguyen *et al.* (2004) [16] and Lacape *et al.* (2005) [6] have used 75 individuals of BC₁F₁ population. There is no specific study relating to the ideal number of individuals in a given population required to establish accurate genetic maps. A segregating population consisting of 50 to 250 individuals is required to construct the linkage map (Mohan *et al.*, 1997) [17], but high resolution fine mapping is possible only with a larger population size. Hence, it was believed that the population size used in this investigation was appropriate. SSR markers have been used for linkage map construction in this report.

SSRs can be used as anchors to combine linkage map produced with different populations to generate more comprehensive genetic linkage map. Out of 391 markers, 141 SSRs revealed (41.43 per cent) polymorphism between KC3 and Suvin which was in accordance with recorded polymorphism of 38.31 per cent (Guo *et al.*, 2007) [18]. This polymorphism rate provided by SSRs is generally regarded as sufficient for constructing a reasonably saturated linkage map. However, during linkage analysis it was found that >60 per cent of markers remained unlinked. This might be attributed to the existence of inter-parental incompatibility alleles, poor chromosomal rearrangements which could be due to narrow marker coverage and lack of informative recombinations (Lin *et al.*, 2005) [2].

The current linkage maps covered approximately 16.5 per cent of genome, since the markers have sparsely distributed. Formation of small and a less number of total linkage groups also indicate that the marker coverage in the genome is not exhaustive and more number of markers needs to be added to the map to represent the entire genome. Similar kind of poor coverage of cotton genome has already been reported. For example, eleven linkage groups were identified from a trispecific F₂ population that spanned 521.7 cM with an average distance of 16.8 cM (Altaf *et al.*, 1997) [19]. Han *et al.* (2006) [20] constructed a linkage map from BC₁F₁ population which contains only six linkage groups. The earlier linkage studies also shown poor coverage of cotton genome due to sparse distribution of markers and less number of total linkage groups (Bolek *et al.*, 2006 [21], Wang *et al.*, 2006 [22], Abdurakhmonov *et al.*, 2007 [23], Guo *et al.*, 2008 [24], Lu *et al.*, 2008 [25]). The sparse distribution of the QTLs in this study is consistent with the observation that a significant proportion of cotton genome comprises of repetitive sequences (Reinisch *et al.*, 1994) [3]. Hence, identification and incorporation of additional SSRs or other kinds of high-throughput markers are required to construct a sufficiently dense linkage map for reasonable QTL analysis. Lacape *et al.* (2005) [6] have published such a map with BC₁ and BC₂ populations.

The key finding of this study was identification of two major QTLs linked to fibre quality traits in the interspecific backcross mapping population. The genomic region flanked by CIR150 and CIR407 was associated with micronaire on chromosome 25 has already been reported with different genetic backgrounds. Contribution of this QTL to phenotypic variance was noticed as 14.7 per cent in this study. This can be considered as major QTL since it represents more than 10 per cent of phenotypic variance

(Collard *et al.*, 2005) [11]. Hence, this QTL has its potential to be used in future cotton improvement programme. Yet another appealing outcome of this study was identification of a major QTL region for 2.5 per cent span length located on chromosome 19, which was not reported by any of the earlier studies. This QTL explained 17.7 per

cent of phenotypic variation. This may be a unique QTL of this population. However, use of this QTL in MAS has to be confirmed with replicated field trials across the environments with the employment of immortal populations such as RILs and NILs.

Table 1: Trait mean values for KC3, Suvin and 62 BC₁F₁ progenies along with broad sense heritability

Traits	Minimum	Maximum	Mean			Heritability
	BC ₁ F ₁	BC ₁ F ₁	KC3	Suvin	BC ₁ F ₁	BC ₁ F ₁
2.5% span length (mm)	24.80	34.10	26.28	36.83	29.27	71.20
Bundle strength (g/tex)	16.60	25.90	22.27	32.89	20.55	67.80
Micronaire	2.50	4.10	4.81	3.53	3.18	62.80
Uniformity ratio	40.00	49.70	48.6	48.9	44.27	54.10
Elongation per cent	4.40	7.80	5.95	7.03	5.99	72.30

Table 2: QTLs detected for fibre quality traits based on interval mapping (QTL Cartographer v 2.5) in 62 BC₁F₁ progenies derived from KC3 (recurrent parent) and Suvin (donor parent)

Chromosome number	QTLs	Marker interval	Population type	Trait	LOD score	R ² (%)
19	Q2.5SL _{19.1}	CIR219-NAU3935	BC ₁ F ₁	2.5 per cent span length	2.6	17.7
25	QMIC _{25.1}	CIR150-CIR407	BC ₁ F ₁	Micronaire	2.5	14.7

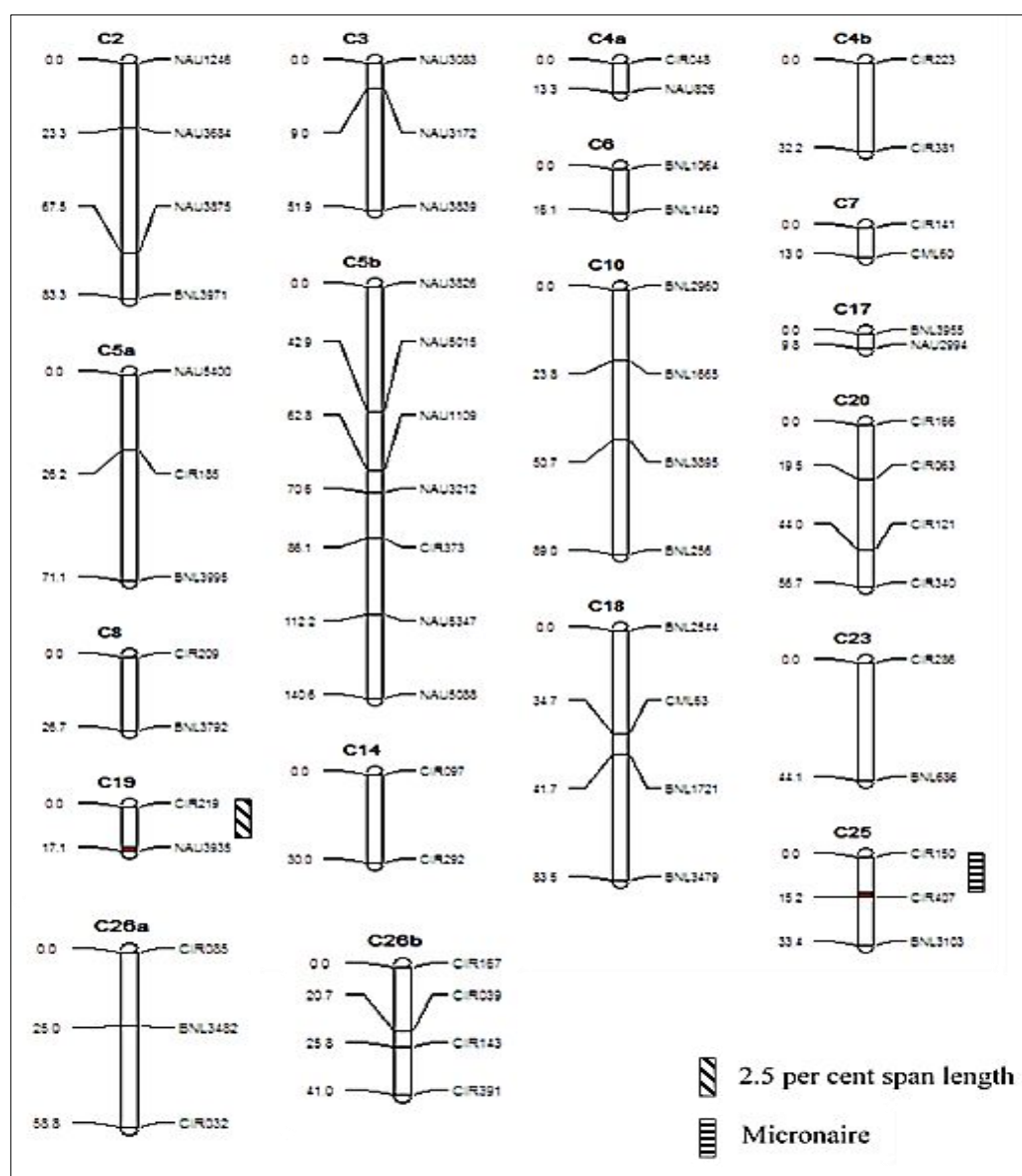


Fig 1: Nineteen linkage groups showing the marker positions and two major QTL regions identified in interspecific BC₁F₁ mapping population

Conclusion

The two major QTLs reported in this study indicate their usefulness to breeders to trace this important fibre quality trait in early growing stage and early segregating generations and thus enormous cost on raising the experiments and manpower can be saved. Hence, markers identified in this study could be useful in future breeding programme to improve fibre quality traits in cotton.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

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