

# Genetic linkage map construction for fibre quality traits in intraspecific upland cotton (*Gossypium hirsutum* L.)

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#### Abstract

The quantitative traits such as fibre length, fibre strength, fineness, uniformity, color and elongation are considered as important fibre quality traits fetches high market price for cotton, thus identification of quantitative trait loci (QTL) for fibre quality traits in cotton (*G.hirsutum*) would be beneficial for cotton fibre yield and quality improvement . Hence the main objective of this study was to identify QTLs for fibre quality traits using an intraspecific mapping population derived from a cross between JKC737 (*G.hirsutum*) and JKC725 (*G.hirsutum*) comprising 180 F<sub>6</sub> – RILs. Population study was carried out for 3867 primer pairs using two parental genotypes (JKC737 & JKC725) of a mapping population developed for fibre quality related traits; only 174 SSR were polymorphic. Owing to a low level of polymorphism between the parental genotypes and a high degree of segregation distortion in recombinant inbred lines, genotypic data of only 120 polymorphic SSR on the mapping population consisting of 180 RIL could be used for construction of a linkage map; 120 SSR loci were mapped on eighteen different linkage groups that covered a total genetic distance of 2883.8cM. Hopefully this map will be enriched with more SSR loci in future and will prove useful for identification of quantitative trait loci/genes for molecular breeding involving improvement of fibre strength and other related traits in cotton.

**Keywords:** QTLs, fibre quality traits, genetic linkage map, marker assisted selection, RIL, polymorphic primers

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#### INTRODUCTION

Improving cotton fibre quality is a major breeding goal for upland cotton in India. The advancement in molecular biology provides new approach like genomics to construct molecular map of key traits that will reveal genetic architecture of traits and helps in the marker assisted selection (MAS) for cotton improvement. Using DNA markers in MAS can untether direction towards robust crop improvement (Burr *et al.* 1983, Tanksley *et al.* 1988, Xu and Crouch 2008) especially complex traits like fibre quality (Kohel *et al.* 2001) through indirect selection of target traits.

Genetic mapping and marker-assisted breeding in all crops require a suitable marker system with increased throughput, decreased cost per data-point and best map resolution (Kumar *et al.* 2007; Gupta *et al.* 2008). The drafted genome of tetraploid cotton (*Gossypium hirsutum*) has just been published, overcoming the sinister task of quality assembly due to highly homologous subgenomes (Li *et al.* 2015, Zhang *et al.* 

2015). Sequencing initiative of G. hirsutum waited for a long time due to large genome size (2.5 Gb) and huge amount of (~80%) repetitive sequences (Hendrix and Stewart 2005). Most of high-density interspecific linkage maps (Fang and Yu 2012, Rong et al. 2004, Yu et al. 2011, Zhao et al. 2012) and intraspecific linkage maps have been developed in cotton. Adding to that, so many QTL mapping studies for fibre quality and yield have been conducted with both interspecific (He et al. 2007, Lacape et al. 2005, 2010, Mei et al. 2004, Yu et al. 2013) and intraspecific populations (He et al. 2011, Liu et al. 2012, Qin et al. 2008, Shen et al. 2006, Sun et al. 2012, Ulloa et al. 2005, Wang et al. 2006, Wu et al. 2009, Zhang et al. 2005, 2009, 2012). All those studies contribute more to understanding of complexity of genetic control of cotton fibre quality and yield.

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Microsatellite DNA markers has been most widely used, due to its ease of use by simple end-point PCR and to high degree of information provided by its large number of alleles per locus (Kumar et al. 2009). Researchers developed SSRs to identify markers correlated with important quality traits: such as fibre vield and quality (Zhang et al. 2005). Chee et al. (2004) highlighted utility of G. arboreum EST sequences in Genbank database for developing PCR-based markers targeting known function genes in cultivated tetraploid cotton. Kumar et al. (2006) demonstrated that searching cotton ESTs in BLAST against Arabidopsis database is both feasible and practical for predicting location of introns in cotton ESTs. Han et al. (2004), Qureshi et al. (2004) and Park et al. (2005) demonstrated EST-SSR markers as a cost-effective strategy for cotton by exploiting EST databases that would facilitate the development of a high-resolution integrated genetic map of cotton for structural and functional study of fibre genes and fibre quality marker assisted selection. Abdurakhmonov et al. (2008) highlighted that SSR and EST-SSR markers associated with fibre development traits have the potential to play a key role in understanding of cotton fibre development and also for development of superior cotton cultivars through marker assisted selection programs.

# MATERIALS AND METHODS

# Plant Materials Used and Experimental Conditions

Two genotypes of *G. hirsutum* (superior JKC725 and inferior JKC737) with respect to fibre quality are used in this study. These cotton varieties are parents of two mapping population developed at R&D Centre, JK Agri Genetics Ltd, Ravalkol, Hyderabad during 2013-2014. The intraspecific mapping population (RIL) of cotton was also generated at the same location.

Briefly, for the generation of intraspecific recombinant inbred lines (RIL) two *Gossypium* genotypes contrasting for fibre quality traits JKC737 and JKC725 were crossed and the resulting  $F_1$  plant was self-pollinated to obtain the  $F_2$  offspring that were further self-pollinated and advanced to  $F_6$  generation using single seed descent method to obtain RILs. An intraspecific mapping population comprising 180 RILs was used for construction of linkage map and further for QTL analysis.

#### **DNA** Isolation

Leaf samples of two different genotypes (JKC737 & JKC725) of *G. hirsutum* were collected from Ravalkol, Hyderabad. Fresh or frozen leaf tissues of cotton plants were used for DNA extraction. Fresh tissue quickly frozen in liquid nitrogen gave satisfactory yields up to 3 months of storage. The protocol was standardized for 2 g samples that can be handled in a 50 ml disposable screw capped tube, while similar conditions on mini

scale using 0.2 g tissues can be scaled down and easily fit into an eppendorf tube. DNA was extracted by modified CTAB extraction method and the DNA concentration and purity of each sample were measured with a UV-Vis spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). Quality of DNA was checked in agarose gel electrophoresis.

## Analysis of RILs with Polymorphic SSRs

Genomic DNA of 180 samples from JKC725 and JKC737 were first screened with SSR markers to identify polymorphic markers between the two mapping parents. Primer sequences for SSR markers are available from the Cotton Microsatellite Database. RILs were developed after F6 generation to get uniform and pure homozygous population. In most cases alleles are distributed in the RIL population in a 1:1 ratio.

A total of 3867 SSRs were used to get the polymorphic primers between the two genotypes of *G. hirsutum*. A total of 174 SSRs were found to be polymorphic. Majority of SSR primers are having a band size of 200bp to 300bp. However, when we ran all the SSRs against the RIL population, 54 SSRs showed segregation distortion (i.e., didn't follow the Mendelian segregation ratio 1:1) accompanied by more than 20% heterozygosity. Those SSRs are removed from further analyses.

## **Construction of Linkage Map**

Linkage map was constructed with the help of **MAPMAKER3** from RIL population generated in our R&D field at Hyderabad. A total of 120 SSRs were used for construction of map. The dataset was prepared in excel spreadsheet and uploaded in MAPMAKER for assignment of SSRs in different linkage groups (at min LOD 3.00, max Distance 50.0). Eighteen groups were generated and few SSRs did not make any groups, and the number of SSRs in each group varies from 2 to 13.

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**Fig. 1.** An intraspecific genetic linkage map of *G. hirsutum* based on RIL mapping population generated by crossing *G. hirsutum* (JKC737 x JKC725). The map of 18 linkage groups were generated with 120 polymorphic genomic SSR markers using MAPMAKER3 at min LOD 3.0 max Distance 50.0



Fig. 1 (continued). An intraspecific genetic linkage map of G. hirsutum based on RIL mapping population generated by crossing G. hirsutum (JKC737 x JKC725). The map of 18 linkage groups were generated with 120 polymorphic genomic SSR markers using MAPMAKER3 at min LOD 3.0 max Distance 50.0

#### **RESULTS AND DISCUSSION**

Cotton marker-assisted selection programme is currently focused on introgression a fibre quality QTL from a Gossypium hirsutum cultivar (JKC725, which produces a very strong, long thin fibre) into a Gossypium hirsutum cultivar (JKC737 early and high yielding). QTLs underlying good fibre quality traits were located in and around 17 different chromosome segments on cotton genome map. Breeders hope to enhance intrinsic fibre quality of JKC725 through the accumulation of these fibre quality-associated QTLs from JKC737. A total of 120 polymorphic markers were used to construct 18 Linkage groups covering a total of 2883.8 cM.

Opportunity for improve our understanding of genetic variability and structure of G.hirsutum genome, a comprehensive PCR-based marker linkage map for fibre quality that covered 70.6% of upland cotton genome was constructed. Recently Zhang et al. (2012) developed a crossing population in upland cotton and constructed a genetic map covered 4184.4 cM, and that approximately spanning 94.1% of the complete tetraploid cotton genome and having 978 simple sequence repeat (SSR) loci. A drafted physical map of D-genome cotton species (Gossypium raimondii Ulbr., D5) has been completed and G. hirsutum genome is being sequenced, which gives functional markers and SSR markers for construction of genetic linkage map and QTL mapping for fibre quality traits to facilitate MAS. In the present study, a genetic map was constructed using an RIL population derived from an upland cotton hybrid and used for tagging cotton fibre guality in upland cotton.

The occurrence of segregation distortion in plants is very common, a process in which genotypic frequencies are skewed from expected violating Mendelian segregation ratios and these deviations can't be evaluated by simple genetic methods (Li et al. 2010, Lu et al. 2002. Song et al. 2005). Segregation distortion is widespread in intra and interspecific crosses (Causse et al. 1994, Lacape et al. 2009, Rong et al. 2004, Ulloa et al. 2002, Yu et al. 2011, Zhao et al. 2012), and is driving force in evolution of species (Taylor and Ingvarsson, 2003). Mangelsdorf and Jones (1926) reported for first time occurrence of segregation in maize, using morphological markers and afterward McCouch et al. (1988) and Pereira et al. (1994) reported segregation skewness in rice, sorghum, and tomato. Many factors like pollen tube competition, pollen killer genes, selective fertilization, abortion, chromosome translocation etc., are major causes of segregation distortion (Li et al. 2007, Luo and Xu 2003, Taylor and Ingvarsson 2003, Zhu et al. 2007).

In this study, genetic map of cotton is developed from JKC737 x JKC725 which will serve as vital genomic resources for fine positioning of important traits, genome organization and function, map-based gene cloning, comparative genomic analyses in cotton. MAS studies unveil that upland cotton has narrow genetic base resulting into low rate of polymorphism among them

(Gutierrez *et al.* 2002, Saha *et al.* 2003, Wendel *et al.* 1992, Van Esbroeck *et al.* 1998). For the efficient utilization of genetic resources from cotton molecular breeding approaches need to be established. This requires better understanding at genomic level which is the key feature. It is therefore a dire need to construct genetic linkage map between upland cotton.

Out of 3867 SSR primer pairs tested, 174 SSR primers (3.5%), detected polymorphism between two genotypes. The polymorphic band size varies from 150bp to 275 bp. In some SSRs more than two polymorphic bands were also found between the two genotypes. More than two alleles were detected with most of the primers, and only polymorphic alleles were considered. The fact that we detected more than two alleles in many SSRs indicates the tetraploid origin of *Gossypium hirsutum*. The allele sizes varied from 100 bp to 400 bp. Most of the SSR primers are bi, tri, tetra and hexanucleotides. A similar low level (2.97%) of SSR polymorphism was reported by Wang *et al.* (2006) who tested 4106 markers in a *G. hirsutum* RIL population for QTL mapping of fibre quality traits.

Out of 174 polymorphic primers 120 polymorphic primers followed the Mendelian segregation in F6 RIL population. A basal level of heterozygosity has been detected in most SSRs with an average of 8.4%. So the population has not yet become fully homozygous. May be another two or three generations is needed for getting 100% homozygous population. This may be due to the polyploidy nature of this species.

Skewed segregation ratios have been reported frequently in cotton (Lacape *et al.* 2003, Mei *et al.* 2004, Ulloa *et al.* 2002, 2005). High frequency of segregation distortion (49–80%) has been observed in inter-specific crosses, most likely due to divergence between species

(Paterson *et al.* 1988). The frequency of distorted ratios was observed in this study. Total 54 SSR primers were discarded due to this distortion.

From linkage map it was found that except 10 SSRs remaining 110 SSRs are distributed in 18 different linkage groups. Group no. 3,6,8,9,11,12,13 shows similarity with previous results. Group14, 15 and 16 are newly formed group having no similarity with previous results. But group no.1,2,4,5,7,10,17 and 18 are ambiguous and consists of both type of chromosomes if compared with previous results. With the help of above linkage map QTLs can be identified for fibre quality in cotton. . In addition to conventional breeding methods, cotton breeders have access to new biotechnology tools such as marker-assisted selection which enable them to create new cotton varieties that are higher yielding and generate a better quality fibre. Varieties can thus be screened directly on the basis of genotype, i.e. from genes contained in the genome, contrary to conventional breeding strategies based on phenotype (physical manifestation of a trait in a plant induced by expression of specific genes).

Understanding the molecular genetics of genome cotton can be important for many reasons. They can foremost serve as a simple model system to study complex quantitative traits, yet only a limited number of genetic maps and QTL studies have been conducted. There is a significant opportunity for further mining the diploid genome with efficient marker systems to facilitate genetic mapping of fibre genes. In the present study, we used SSR markers to generate a framework genetic map of cultivated diploid cottons. The genetic map was used to identify QTL linked to fibre traits.

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