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# Genetic improvement of *Gossypium arboreum* L. using molecular markers: Status and development needs

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The genus *Gossypium* has 45-50 species, of which only four (two allotetraploids and two diploids) enjoy the status of cultivated cotton. Among the four cultivated ones, *Gossypium arboreum* holds a special place because of the inherent ability to withstand drought, salinity and remarkable resistance to sucking pests and leaf curl virus. However, the species suffer with poor fiber quality traits, low yield and has certain undesirable plant and boll features. Improvement of fiber quality traits of *G. arboreum*, without disturbing its unique characteristics, has been a long sought after dream of scientists world over. For conservation, evaluation and documentation of existing accessions, gene banks of diploid cultivated cotton has been established by different countries, of which India holds the highest number of *G. arboreum* collections. Traditional breeding efforts made to improve fiber quality of *G. arboreum* have met with limited success due to paucity of polymorphic phenotypic markers and polygenic nature of the desired traits. Genetic engineering approach is highly genotype dependent and much success has not been achieved by this way also. Molecular breeding approach, based on the strength of breeding with high polymorphic nature of molecular markers, has yielded significant results in the improvement of *G. arboreum*. A number of molecular markers have been developed and used in various cotton improvement programs but dominantly these efforts have been made for the allotetraploid species *G. hirsutum*. Some efforts have been made for generating fingerprint database of *G. arboreum* germplasm, though much more efforts are needed in this direction. Linkage maps of *G. arboreum* have been generated using different markers systems which has enabled the mapping of gene/QTLs of desired traits on the chromosome of *G. arboreum*. More research inputs need to be devoted to produce consensus and saturated genomic maps, if the aim of 'breeding by design', by retaining suitable features and improving the undesirable ones, is to be realized for *G. arboreum*.

**Key words:** Asiatic cotton, molecular breeding, cotton fiber quality, molecular breeding, genetic diversity.

## INTRODUCTION

Cotton is the world leading natural fibre crop on which the textile industries worldwide are largely based on. It is also an important oilseed crop contributing significantly to bio-energy production. Cotton occupies a pivotal position in

the world economy, and very often, it is known as 'white gold'. The term cotton is used to describe cultivated species of the genus '*Gossypium*' (family Malvaceae) (Wendel et al., 1992; Wendel and Brubaker, 1993;

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Dongre and Kharbikar, 2004; Esmail et al., 2008), which has 45-50 species, 40-45 being diploid ( $2n=2x=26$ ) and 5 being allotetraploid ( $2n=4x=52$ ). Spinnable fibers are obtained from two allotetraploid (*Gossypium hirsutum* and *Gossypium barbedense*) and two diploid (*Gossypium herbaceum* and *Gossypium arboreum*) species and hence only these four species enjoy the status of cultivated cotton. The diploid species are popularly called as old world, or desi cotton (in India and Pakistan), while the allotetraploids species are commonly known as new world cotton (Wendel et al., 1992). The present paper is an attempt to highlight the strengths and weaknesses of *G. arboreum* germplasm and to discuss present status and development need of various improvement programs.

### Domestication of *G. arboreum*

There is evidence of its cultivation as old as 2000 BC by the Harapan civilization of the Indus Valley for the cotton textiles. With time, *G. arboreum* germplasm was dispersed and domesticated in different regions leading to the development of different races viz. indicum, burmanicum, cernuum, sinense, bengalense, soudanase. Over the years of cultivation, various ecotypes of each race have been identified in different parts of Indian subcontinent. Bengal Desi, of race 'bengalense', is popularly grown in North India and Pakistan and is found to be highly productive but yielding short (12-20 mm length) and very coarse fibers (>5.5 micronaire). Various ecotypes of race 'indicum' have developed to grow successfully in the diverse harsh environmental conditions of Tamil Nadu, Gujrat, Karnatka and Andhra Pradesh. One ecotype (Gharo cotton) of race 'cernuum' is found to yield very big balls and is popularly cultivated in North-Eastern regions of India and adjoining parts of Bangladesh (Kulkarni et al., 2009).

### Characteristics features of *G. arboreum*

*G. arboreum*, also called true cotton, is a species native to Indian sub-continent. *G. arboreum* has certain inherent qualities like the ability to withstand drought and salinity (Maqbool et al., 2010; Tahir et al., 2011) making it suitable for low input conditions. Many studies demonstrated that the asiatic cotton is tolerant and shows remarkable resistance to several pests and disease, including bollworms (Dhawan et al., 1991), aphids and leafhoppers (Nibouche et al., 2008), rust, fungal (Wheeler et al., 1999) and viral (Mehetre et al., 2004; Akhtar et al., 2010) diseases. The reasons for resistance to sucking pests are presence of lower palisade layer, relatively higher distance between lower epidermis of midrib and phloem, and densely arranged midrib cortex cells in *G. arboreum* plants. In fact, presently *G. arboreum* is being

used as donor species in introgressive breeding to improve tetraploid cotton, especially for disease resistance and insect tolerance (Ansingkar et al., 2004; Kulkarni, 2002). Natural *G. arboreum* fibers display various colours (e.g. white, off-white and tan) also and some of the accessions produce fibers with high strength (Mehetre et al., 2003).

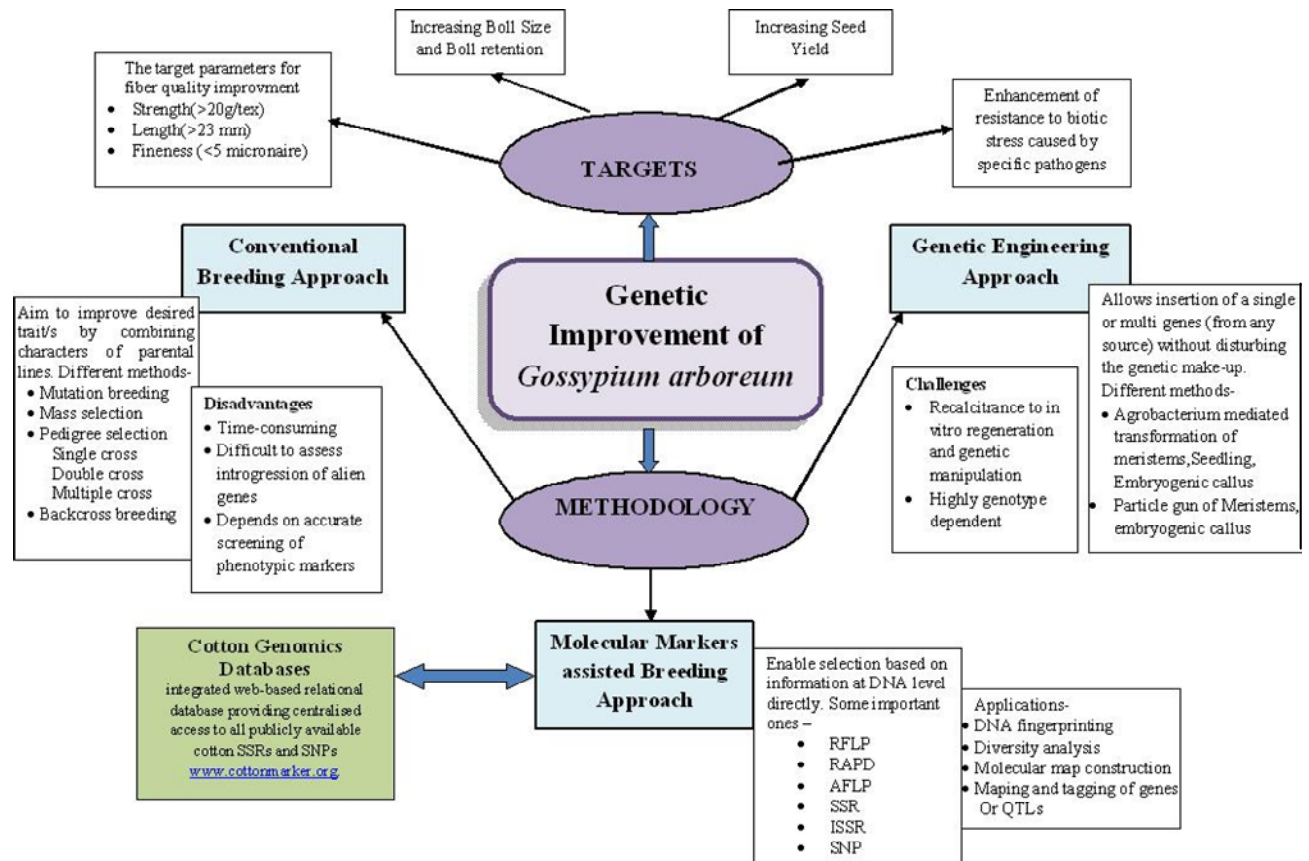
### Present cultivation scenario of *G. arboreum*

Till the 1950s, a major part of cotton area of Old World (Asia and Africa) was occupied by diploid cultivated cotton (Kulkarni et al., 2009). After that with increase in global trade, American upland cotton and Egyptian/sea-island cotton viz. *G. hirsutum* and *G. barbadense* respectively, replaced the diploid cotton. At present, the area of diploid cotton cultivation in India is around 25% (17% *G. arboreum* and 11% *G. herbaceum*), while for rest of old world countries it has come to a very low scale (Kulkarni et al., 2009). The major cause for this change was due to fibre, boll and plant features of diploid cotton. The primary fibre properties affecting textile manufacturing and end product quality include fibre length and uniformity, strength, elongation, fineness and maturity (Chee and Campbell, 2009). Diploid cultivated cotton are characterized by short (<23 mm fibre length), coarse (>5.0 micronaire) and weak (<20 g/tex at 3.2 mm gauge) fibres, which are not suitable for spinning by mechanized textile technology (Kulkarni et al., 2009) (Figure 1). The lanky plant structure with indeterminate growth and small bolls with less locule retention are major hurdles in cotton harvesting, impairing the economics of cultivation (Figure 1). Still, poor and marginal farmers prefer *G. arboreum* because of the characteristics features discussed above.

Genetic improvement of *G. arboreum* for better fiber quality traits, without disturbing its unique characters, is needed to promote its cultivation. Such efforts will boom the fiber industry and go a long way for strengthening the world economy. In the following section, an effort is being made to explore the various on-going efforts and approaches for genetic improvement of *G. arboreum* so as to analyze the strengths, achievements made so far as well as to identify the challenges being faced by these methods. Further, current status of cotton molecular marker technology and the scope, potential and future perspective of this technology for the genetic improvement of *G. arboreum* are discussed in light of suitable examples.

### Improvement of *G. arboreum*: Status and challenges

The success of genetic improvement program of any crop is based on the identification of well defined targets and adoption of reliable methodology. Although, diploid



**Figure 1.** A diagrammatic description of various targets for genetic improvement of *G. arboreum* along with various methodologies adopted for this, with characteristics features of each.

cultivated cottons are grown in nearly eleven countries (India, Pakistan, China, Burma, Nepal, Cambodia, Malaysia, Indonesia, Namibia, Vietnam, Laos), organized genetic improvement programs are dominantly made in India. Diploid cotton improvement projects are a part of All India Coordinated Cotton Improvement Project (AICCIP) and supported by Technology Mission on Cotton (TMC), Mini Mission I and Rainfed Cotton Agroecosystem (RCPS) programs of the National Agriculture Technology Project ([www.cicr.nic.in](http://www.cicr.nic.in)). The research projects designed for improvement of *G. arboreum* are generally targeted at: (i) Improvement of fiber quality traits, like length and fineness (ii) Increase in the boll size (iii) Increase in yield (iv) enhancement of resistance to various biotic stress- though *G. arboreum* is resistant to sucking pests and immune to leaf curl viral disease, certain other disease and pests affect it. Grey mildew (*Ramularia areola* Atk), Leaf spot (*Alternaria macrospora*) and Fusarium wilt are important disease while bollworms (*Helicoverpa armigera*, *Earias vitella* and *Pectinophora gossypiella*) are insect pests causing economic damage to *G. arboreum* (Kulkarni et al., 2009). A significant level of variability, for the desired characters, exists in the germplasm of *G. arboreum*, indicating good

potential for genetic improvement. Appreciable genetic variability has been observed in the *G. arboreum* germplasm for yield and yield components (Singh and Singh, 1984; Kumar and Rajmani, 1994), fiber properties (Singh, 1986), boll size (Kulkarni et al., 2009) and locule retention (Singh and Nandeshwar, 1983). Various improvement efforts have been made to exploit this genetic variability using traditional plant breeding methods based on morphological markers. Traditional approaches to increase the seed yield have been quite successful; varieties released during 1970s and 1980s had less seed cotton yield potential (1600 to 1800 kg/ha) compared to varieties released in 1990s and 2000s (e.g. LD 327, HD 107 and RG8 (2500-2600 kg/ha), a hybrid AAH1 (3900 kg/ha) in north Indian irrigated conditions (Singh, 1998; Lather et al., 2001). Efficient enhancement of fiber properties and boll feature of *G. arboreum* has also been reported through *G. hirsutum* gene introgression program (Kulkarni et al., 2003). Some successful breeding attempts were also made for improving the locule retention of *G. arboreum* bolls; CINA36 is an improved line which has been registered for good locule retention (Kulkarni et al., 2009).

The various conventional breeding approaches are

generally very time consuming (Figure 1); generally takes five to six generations for transfer of a trait, within a species, into the high yielding locally adapted cultivars and a large number of progenies have to be planted to select the plants with appropriate combinations of traits. After that, the improved lines so developed have to go through a number of multi-location trials before a variety could be identified for cultivation by the farmers. In way, a total of 7-10 years or more are involved from the beginning to the end of the project. Further, sometimes it is difficult to combine more than one trait in a single breeding experiment, and improvement in one aspect is many-a-times found to be associated with undesirable characteristics of another aspect e.g. 'Fiber improvement' breeding programs which used 'indicum' as source for long and fine fibers, suffered with small boll size problem and when 'cernuum' was used to improve boll size it led to undesirable fiber properties (Kulkarni et al., 2009). Breeding efforts in fiber quality have been very effective, which used mostly 'indicum' source for long and fine fiber. Mass selection generated a wide range of exploitable genetic variation for fiber properties but boll size remained small (Rao et al., 2004).

Genetic transformation is one of the powerful techniques offered by biotechnology for genetic improvement of crop plants with desired traits. The most important application of this has been the development of Bt cotton which also happens to be first genetically modified crop marketed successfully and still very popular. *In vitro* regeneration of all the four cultivated species of cotton has been a difficult goal to achieve because morphogenic response is highly genotype dependent (Trolinder and Xhixian, 1989; Cousins et al., 1992; Rajasekaran et al., 1996) (Figure 1). Much of the genetic transformation work in cotton has been done for tetraploid species and for this also, foreign gene to be introduced is generally put into Coker (highly responsive to *in vitro* regeneration) followed by transferring into elite cotton cultivars through conventional breeding method like back cross breeding which take longer time. Very few studies have been carried out for genetic transformation in *G. arboreum*. Generally *G. arboreum* genotypes are more tolerant to insects than *G. hirsutum*, but in case of severe outbreaks economic losses are observed. Further there are certain genotypes which have desired fiber traits but suffer with high susceptibility to insects attack e.g. DLSa-17 which is long linted but susceptible to all lepidopteran pests. As there is no germplasm accession that provides durable resistance to bollworms, the transgenic approach offers the solution. An effort has been made to transfer cry 1F gene into DLSa-17 to make it insect resistant; transformants were obtained at low frequency (Sangannavar, 2008). Various factors affecting transformation efficiency of shoot apices of *G. arboreum* cultivar LD 694, using *Agrobacterium* strain GV3101 carrying plasmid pPZP200 vector with cry1AC gene, have also been reported (Sanghera et al., 2011). In

addition to developing bioengineered insect resistant desi cotton, other traits like lint yield, fiber quality, seed oil content, resistance to fungal and viral infection also need to be addressed by genetic transformation. The prerequisite for this is the development of protocols which overcome the recalcitrance of cotton tissues to genetic manipulation and *in vitro* regeneration, which seems to be a far dream though. So if genetic transformation is compared with breeding approach, latter seems to be more reliable and stable approach for cotton with wider applicability.

The only drawback of breeding approach is dependence on less polymorphic morphological markers. With the advent of molecular marker technology, a new era of breeding has emerged. DNA marker technology enables the plant breeders to select desirable plants directly on the basis of genotype instead of phenotype. DNA marker technology has enabled the development of a sufficiently large number of genetic markers to accommodate the needs of modern plant breeding, also termed as 'Molecular breeding'. With the use of molecular marker technology, breeding efforts have become more specific, less time consuming and more yielding in terms of the target level achieved. In the coming section a brief discussion has been made about different types of molecular markers developed and applied in cotton followed by different applications these molecular markers offer for genetic improvement of *G. arboreum*.

### Cotton molecular markers: Types and database

Since the last three decades, a lot of efforts have been put in for developing various types of DNA markers, each having a differential set of advantages for any particular applications. Application of this technology towards cotton improvement programs started in the 1990s, Meredith (1992) in a study of heterosis and varietal origins reported the first restriction fragment length polymorphism (RFLP) evaluation in upland cotton. First detailed RFLP map of cotton with 41 linkage groups was developed by Reinisch et al. (1994). Since then, numerous types of marker systems have been developed and applied to cotton improvement programs. In the coming section, a brief overview is made about various types of molecular markers being developed and used for cotton genomics followed by a discussion about cotton marker database.

### Types of molecular markers

The cotton genome is large and complex with 26 chromosomes, which require identification of a large number of evenly spaced DNA markers for significant applications (Park et al., 2005; Qureshi et al., 2004). A number of molecular markers have been developed and used for cotton (Figure 1).

### Restriction fragment length polymorphism (RFLP)

It is hybridisation based technique in which organisms are differentiated by analysis of patterns derived from cleavage of their DNA by restriction enzymes. The main steps involve isolation of DNA, digestion with restriction enzymes, separation of restriction fragments by agarose gel electrophoresis, transfer of fragments to nylon membrane, hybridization with probes, and scoring of polymorphism by autoradiography. RFLP markers are reliable, reproducible and co-dominant making them ideal tool for genome mapping and gene tagging experiments, though the technique is time-consuming, cumbersome and not amenable to automation (Table 1). RFLPs have been widely used in gene mapping studies because of their high genomic abundance, ample availability of different restriction enzymes and random distribution throughout the genome (Neale and Williams, 1991). RFLPs have played a strategic role in initiating and pushing the research work in cotton genomics (Rahman et al., 2009) and a number of researchers have applied this technique to various *Gossypium* spp. for different purposes (Reinisch et al., 1994; Jiang et al., 2000; Mei et al., 2004; Ulloa et al., 2005). However, at present, RFLPs are not popular in cotton genome studies because of low ability to detect polymorphism in cotton compared to other plant taxa (Brubaker and Wendel, 2000).

### Random amplified polymorphic DNA (RAPD)

It is a polymerase chain reaction (PCR) based technique, based on enzymatic amplification of target or random DNA segments with arbitrary primers. Polymorphism is obtained because of sequence variation in the genome for primer binding sites, making RAPDs as dominant marker. RAPD marker system is easy to carry out, needs no prior sequence information, requires very less amount of DNA and is amenable to automation, however, the technique suffers with low reproducibility (Rafalski, 1997) and some more disadvantages mentioned in Table 1. The poor reproducibility of RAPD profiles within and between laboratories can be attributed to various factors like quality of DNA, composition of PCR reaction mixture, nature of DNA polymerase and the skills of the working person, to quote a few. RAPDs have been used for diversity, genome mapping and phylogenetic studies in cotton (Rahman et al., 2002b; Zhang et al., 2002; He et al., 2007; Rahman et al., 2008b; Rana and Bhat 2004). RAPDs are not popular for genetic mapping and gene tagging studies in cotton because of their low informativeness and non-locus specific nature.

### Amplified fragment length polymorphic (AFLP)

It is a technique which combines reliability of RFLP with the ease of RAPD (Vos et al., 1995). The process

involves three simple steps: (i) restriction of genomic DNA and ligation of oligonucleotide adaptors, (ii) pre and selective amplification of restriction fragments, and (iii) gel analysis of amplified fragments. The polymorphic fragments are detected as present or absent making it a dominant marker system. The technique can be automated and allows the simultaneous analysis of many genetic loci per experiment (Table 1). AFLP produces more polymorphic loci per primer than RFLPs, SSRs or RAPDs (Maughan et al., 1996). The availability of many different restriction enzymes and corresponding primer combinations provide a great deal of flexibility, enabling the direct manipulation of AFLP fragment generation for defined applications. The AFLP technique has been extensively used in various cotton improvement programs like phylogenetic studies (Iqbal et al., 2001), linkage and quantitative trait loci (QTL) mapping (Jixiang et al., 2007; Mei et al., 2004; Hawkins et al., 2005; Altaf et al., 1997), genetic diversity studies (Abdalla et al., 2001; Rana et al., 2004a; Zhang et al., 2005) and map saturation studies (Lacape et al., 2003; Zhang et al., 2005).

### Simple sequence repeats (SSRs)

These are also commonly called as microsatellites and were first described in humans (Litt and Luty, 1989). These are short tandem repeats of 2-8 nucleotide motifs. The repeat number of core nucleotide sequence varies from a few to hundreds of times at many independent loci. The SSRs are flanked by unique sequences which have remained conserved between the members of a gene pool during the course of evolution. PCR amplification of SSRs is done by using primers complementary to the flanking regions. The length of the amplified product varies according to the number of repeated motif (Ellegien, 1993). SSRs are found highly polymorphic (Powell et al., 1996) and the polymorphism is due to either slippage of DNA polymerase during replication or unequal crossing over, resulting in differences in copy number of the core nucleotide sequences (Rahman et al., 2002a). Because of their abundance in the genome and codominant nature, microsatellites are considered ideal markers in gene mapping studies (Han et al., 2006). SSRs are highly reliable, allow exchange of data among laboratories and are more robust than RAPD and AFLP (Table 1). Expansion and contraction of SSR repeats in genes of known function can be tested for association with phenotypic variation or, more desirably, biological function (Ayers et al., 1997).

In cotton genome, one microsatellite on an average per 170 kb of genomic DNA has been reported (Zhao et al., 1994), making them a good tool for genome mapping. Out of 10000 SSRs containing genomic fragments isolated from *G. hirsutum*, 588 were sequenced to identify SSRs and primers have been designed for 307 of these (Reddy et al., 2001). SSRs have been used in

**Table 1.** A summarized description of some important molecular markers used commonly in cotton breeding programs.

S/No	Features	RFLP	RAPD	AFLP	SSRs	ISSR	SNPs
1.	DNA Required ( $\mu$ g)	10	0.02	0.5 to 1.0	0.05	0.02	0.05
2.	PCR based	No	Yes	Yes	Yes	Yes	Yes
3.	No. of polymorphic loci analysed	1-3	1.5-50	20-100	1-3	1.5-50	1
4.	Ease of use	Not easy	Easy	Easy	Easy	Easy	Easy
5.	Amenable to automation	Low	Moderate	Moderate	High	Moderate	High
6.	Reproducibility	High	Unreliable	High	High	Moderate	High
7.	Need of sequence data	Not required	Not required	Not required	Required	Not required	
8.	Level of polymorphism	Low	Low-Moderate	Low-Moderate	High	Low-Moderate	High
9.	Dominance	Co-dominant	Dominant	Dominant	Co-dominant	Dominant	Co-dominant
10.	Interspecific transferability	Moderate-high	Low-Moderate	Low-Moderate	Low-Moderate	Low-Moderate	Low-Moderate
11.	Utility in marker assisted selection	Moderate	Low-Moderate	Low-Moderate	High	Low-Moderate	High
12.	Cost and labour involved in generation	High	Low-Moderate	Low-Moderate	High	Low-Moderate	High
13.	Advantages	-High genomic abundance -Can be used in plants reliably -Needed for map based cloning	-High genomic abundance -No radioactive labeling -Relatively faster	-High genomic abundance -Can be used across species -Useful in preparing contig maps	-High genomic abundance -No radioactive labeling -Multiple alleles	-High genomic abundance -Require low quantity of DNA	-High throughput automation
14.	Disadvantages	-Need large amount of good quality DNA -Need radioactive labeling -Cloning and characterization of probe are required	-Cannot be used across species	-Very tricky due to changes in patterns with respect to materials used -Need to have very good primers	-Cannot be used across species	-Non-homology of similar size fragments	-High costs

cotton genomics for studies like phylogenetics and diversity analysis (He et al., 2007; Lacape et al., 2007), linkage mapping (Yu et al., 2005), gene tagging and QTL mapping (Karaca et al., 2002; Zhang et al., 2003), association mapping (Kantartzi and Stewart, 2008).

### Inter simple sequence repeats (ISSRs)

ISSRs were developed by modifying the SSR marker technology. The modification involved the

use of primers complementary to SSR motif itself instead of the flanking sequence thus ruling out any prior sequence information (Wolfe et al., 1998). The procedure was first applied to fungi (Meyer et al., 1993) and was found to work successfully with plants (Gupta et al., 1994) as well as animals (Perring et al., 1993).

Amplification with ISSR primers result in DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. ISSR analysis is reported to be

technically simple, highly reproducible and capable of revealing high polymorphism (Rahman et al., 2009). ISSRs have been reported as quite useful markers for revealing polymorphism in cotton genotypes (Liu and Wendel, 2001).

### Single nucleotide polymorphism (SNPs)

SNPs are a novel class of DNA markers that has emerged recently and have become highly

preferable in genomic studies. The SNPs arise due to change in a single nucleotide position (point mutation) by various genetic phenomena. SNPs are highly abundant making them suitable markers for generating high density genetic maps. SNP markers are co-dominant, distributed normally and sometimes associated with morphological changes (Lindblad-Toh et al., 2000). SNP analysis is useful for cultivar discrimination in crops where it is difficult to find polymorphism. These DNA markers have also been used in cotton for fingerprinting (Rahman et al., 2002b, 2008b), linkage map construction (Reinisch et al., 1994; Zhang et al., 2002; Lacape et al., 2003; Mei et al., 2004; Rong et al., 2004), gene mapping (Shappley et al., 1998; Ulloa and Meredith, 2000) and genetic diversity studies (Rahman et al., 2002b, 2008b).

### Cotton markers database

The cotton marker database (CMD) is an integrated web-based relational database providing centralised access to all publicly available cotton microsatellites and single nucleotide polymorphisms ([www.cottonmarker.org](http://www.cottonmarker.org)). CMD is initiated and funded by Cotton Incorporated. This database also displays data for various microsatellite projects that have been screened against a standardised panel of core germplasm including 12 diverse genotypes selected from cultivated and exotic cottons. Various data mining tools like SSR server, BLAST server, FASTA server, CAP3 server and CMap are accessible at CMD. Hosting of web and database for the CMD is provided by Bioinformatics and Chemical Informatics (BCI) group at School of Computing, Clemson University and Computational resources are provided by Clemson Computing and Information Technology (CCIT). This collection of all publicly available cotton SSR and SNP markers into a centralised, readily accessible web-enabled database provides a more efficient utilization of molecular marker resources and will help to accelerate basic and applied research in molecular breeding and genetic mapping in *Gossypium* species.

### Applications of molecular markers in *G. arboreum* improvement programs: Generating a DNA fingerprint database of the germplasm, existing worldwide

Different countries have established Gene Banks of diploid cultivated cotton for conservation, evaluation and documentation of existing accessions. In India, Central Institute of Cotton Research (CICR), Nagpur, is maintaining a global germplasm collection of diploid cultivated cotton; it holds the highest number of collections belonging to *G. arboreum* (1870) (Anonymous, 2005). The French cotton germplasm collection, preserved in Centre de Cooperation Internationale en Recherche Agronomique pour le

Development (CIRAD), Montpellier, maintains 69 accessions of *G. arboreum* (Dessauw and Hau, 2006). The United States of America (USA) maintains 1730 accessions of *G. arboreum* at Southern Plains Agricultural Research Center (SPARC), Crop Germplasm Research Unit, College Station, Texas (Anonymous, 2005). Likewise, nearly 369 accessions are maintained at Chinese Academy of Agriculture Science, Nanjiang, China (Liu et al., 2006). Systematic race-wise characterization of *G. arboreum* germplasm, existing worldwide, has been difficult due to overlapping morphological traits and non-availability of race specific phenotypic markers. The DNA fingerprint database will help in right documentation of the germplasm.

Further, every country has some policy to carry out various germplasm expeditions of different crops/plants for sampling the existing variability. Such programs are also being conducted for exploring the germplasm of *G. arboreum* and variability is recorded based on morphological characters only, which is unable to give the real picture due to poor polymorphic index. Generation of DNA fingerprint of a newly explored local cultivar and matching it with the existing fingerprint database would help to make a right entry for it.

Liu et al. (2006) used SSR markers to create DNA fingerprint database of 39 *G. arboreum* L. genotypes of China. Of the 358 microsatellite markers analyzed, 74 primer pairs detected 165 polymorphic DNA fragments and 12 genotypes could be fingerprinted with one or more SSR markers.

### Genetic diversity analysis

Genetic diversity is the basis for genetic improvement of any crop. Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety, or population or species (Brown, 1983). The knowledge of genetic variation between and within populations of *G. arboreum* is desirable not only to establish a theoretical basis for conserving the Asiatic cotton germplasm resources but also to target and improve certain ideal characteristics such as fibre quality and various plant features for exploiting these germplasm in modern cotton productions. There exist only a few studies for genetic diversity analysis of *G. arboreum* using molecular markers. Abdalla et al. (2001) used 16 AFLP primer combinations on 3 diploid species and 26 allotetraploid species of genus *Gossypium* and observed genetic similarities, among all taxa, ranging from 0.21 (between the diploid species *G. arboreum* and *G. raimondii*) to 0.89 (within *G. barbadense*). Rana and Bhat (2004) used RAPD markers to assess the genetic relationship among 30 genotypes of *G. arboreum*; of 45 primers surveyed, 63% were found polymorphic which revealed genetic similarity in the range of 47.05 to 98.73%. The results indicated a close genetic relationship

among the cultivated genotypes indicating a narrow genetic base.

Guo et al. (2006) studied the genetic diversity of selected *G. arboreum* accessions collected from different regions of China using SSR markers and observed that largest number of alleles and maximum number of polymorphic loci was present in the A03 linkage group. The polymorphism information content for the 22 polymorphic microsatellite loci varied from 0.52 to 0.98, with an average of 0.89. Genetic diversity of 19 elite genotypes of diploid and tetraploid cotton has been carried out by Dongre et al. (2007) using SSR and ISSR markers, the similarity coefficient obtained ranged from 0.59 to 0.90 and 0.59 to 0.93, respectively, thus suggesting considerable genetic variation between the selected genotypes.

Kantartzi et al. (2009) studied genetic diversity in 96 accessions of *G. arboreum* L. using genomic and EST-derived microsatellite markers. They selected a set of 25 SSR primers based upon high level of informativeness and production of clear PCR bands on agarose gel. The 25 SSR loci were found to reveal 75 allelic variants (polymorphism) ranging from 2-4 alleles per locus. RAPD markers have also been used to study genetic diversity in 20 selected genotypes of *G. arboreum*; of the 40 primers screened, 20 failed to amplify any genotype, 11 showed good band profile in few genotypes while remaining nine gave amplification in all the 20 genotypes (Deosarkar et al., 2010). Out of the total amplified products, 52 bands showed 70.58% polymorphism, while remaining products were monomorphic across the genotypes. Dongre et al. (2011) studied genetic diversity of 16 cultivars of *G. arboreum* using 20 RAPD primers and 24 SSR primers. Fifteen selected polymorphic RAPD primers produced a total of 178 fragments, of which 112 fragments were found to be polymorphic, resulted in 65.14% polymorphism. Twenty selected polymorphic SSR primers produced a total of 64 alleles, of which 41 were found to be polymorphic, resulting in 69.50% polymorphism. More such studies are needed to investigate genetic diversity among the existing germplasm of *G. arboreum*.

### Construction of molecular maps via linkage or association mapping techniques

Linkage maps shows the relative positions of genetic markers along a chromosome that is determined by the recombination frequency during crossover of homologous chromosomes. The mapping of functional genes plays an important role in studies of genome structure, function, and evolution, as well as allowing gene cloning and marker-assisted selection to improve agriculturally important traits. Many linkage maps of tetraploid cotton have been constructed using different molecular markers and mapping populations (Reinisch et al., 1994; Ulloa et

al., 2002; Lacape et al., 2003; Rong et al., 2004). Unfortunately, molecular marker technology has not been applied extensively to map the genome of *G. arboreum*. Only a few reports are present for genetic mapping in *G. arboreum*. To increase the numbers of microsatellites available for use in constructing a genetic map, and facilitate the use of functional genomics to elucidate fiber development and breeding in cotton, sampling of microsatellite sequences from expressed sequence tags (ESTs) transcribed during fiber elongation in the A-genome species *G. arboreum* was done to evaluate their frequency of occurrence, level of polymorphism and distribution in the At and Dt subgenomes of tetraploid cotton (Han et al., 2004). There are only a few reports available for linkage mapping of *G. arboreum* genome. Han et al. (2004) published a genetic map ((TM1 X Hai7124) X TM1) based on 99 SSR markers developed from fiber ESTs of *G. arboreum*. A total of 111 loci detected with these 99 EST-SSRs integrated with 511 SSR loci and two morphological marker loci assorted to 34 linkage groups containing 2 to 41 markers each, covering 5644.3 cm with an average intermarker distance of 9.0 cm. Intraspecific genetic linkage map of the A-genome diploid cotton was constructed with newly developed SSR markers using 189 F<sub>2</sub> plants derived from the cross of two Asiatic cotton cultivars (*G. arboreum* L.) 'Jianglingzhongmian' × 'Zhejiangxiaoshanl'ushu' (Ma et al., 2008). For this study, 268 pairs of SSR primer with better polymorphism were picked out of 6092 pairs which generated a total of 320 polymorphic bands. Linkage map was generated with Join Map 3.0. The total length of the map was 2508.71 cm and average distance between adjacent markers was 9.40 cm. Comparisons among the 13 suites of orthologous linkage groups revealed that the A-genome chromosomes are largely collinear with the At and Dt subgenome chromosomes. Construction of intraspecific genetic linkage map of *G. arboreum* with SSR and RAPD markers and using 180 F<sub>2</sub> plants derived from cross of two cultivars- Ravi X Entry-17 of *G. arboreum*, was presented by Shaheen et al. (2013). Another step after construction of linkage map is QTL analysis for identifying/mapping gene of desired traits. Shaheen et al. (2013) mapped 7 QTLs, including 5 for productivity traits and 2 for fiber traits, in the intraspecific *G. arboreum* linkage map prepared by them. However, identification of map position of a QTL is only the first step for planning a MAS (marker assisted selection) program. After a QTL has been identified, several other things need to be addressed like-(i) Will the QTL work in other genetic background, (ii) Will the QTL have same phenotypic effects in other genetic backgrounds, (iii) is the QTL linked to other undesirable traits, (iv) Will the QTL-marker linkage relationship hold in different generations, (iv) what is the cost and efficiency of applying MAS compared to phenotypic selection alone? (Chee and Campbell, 2009).

Association mapping, based on linkage disequilibrium



(LD) where collection of cultivars, lines, or landraces are genotyped with densely spaced markers, is emerging out as a better technique of mapping (Sorkheh et al., 2008; Mackay and Powell, 2007). There are number of reasons for this, firstly, in the population a broader genetic variation in a more representative background is available. It means that one is not limited to marker and trait loci that happen to differ between two parents (Kraakman et al., 2006). Secondly, LD mapping may attain a higher resolution, because of the use of all the meiosis accumulated in the breeding history. Thirdly, historic phenotypic data on cultivars can be used to link markers to the traits, without the need for new trials with special mapping populations (Sorkheh et al., 2008).

In fact, association mapping was first successfully used for identification of alleles at loci contributing to susceptibility to human diseases (Goldstein et al., 2003). Later on this approach was applied to different plant species as well such as wheat (Bresseghele and Sorrells, 2006), rice (Agrama et al., 2007), barley (Kraakman et al., 2006), maize (Stich et al., 2005) etc. In cotton, association studies are very limited; LD has been measured only in a collection of tetraploid *G. hirsutum* lines (Abdurakhmonov et al., 2007). There is a single report on association analysis of fiber traits in *G. arboreum* accessions (Kantartzi and Stewart, 2008), analysing 56 *G. arboreum* germplasm accessions introduced from nine regions of Africa, Asia and Europe for 8 fiber characters. Genotyping was carried out with 98 SSR markers. Population structure analysis identified six main clusters for the accessions which corresponded to different geographic regions, indicating agreement between genetic and predefined populations. The general linear model method was used to disclose marker trait association and these associations were investigated by fitting single marker regression models for phenotypic traits on marker band intensities with correction for population structure. More studies are needed in *G. arboreum* germplasm and to carry out association analysis between quantitative traits and molecular markers which will also account for the effect of population structure.

### Future perspective

*G. arboreum* is a poor man crop with low input conditions. The genetic improvement in desirable traits of *G. arboreum* will definitely gear up the economy of poor farmer as well as the whole cotton industry. Mapping of genes/QTLs of economically important traits in *G. arboreum* (diploid with AA genome) will also be a great help for cracking similar things in comparatively complex genomes of *G. hirsutum* and *G. barbadense* (tetraploid, AADD genome). Much of the initial years of molecular genetics research into cotton has been devoted to developing core infrastructure facility like polymorphic markers, discrete mapping populations and extensive

linkage maps. This has given an insight about the number and locations of QTL associated with desirable traits. With the advancement made so far and continuous advancement of molecular technology, the coming years will definitely give a clearer picture of the genetic basis of various desirable traits like cotton fiber quality, drought resistance, biotic resistance etc. Molecular breeding is definitely having a bright future in cotton and MAS is going to make an effective complement to phenotypic screening in the future genetic gain for desirable traits.

### CONCLUSION

The Asiatic cotton grows easily in dry land as well as in saline conditions and has inherent resistance to several abiotic and biotic stresses, making it a low input crop. However, the fibers produced by *G. arboreum* are in low yield and of poor quality (in terms of mechanised spinning). A huge germplasm of *G. arboreum* exists worldwide. Well defined and well planned efforts are needed to explore the existing resources for the improvement of fiber quality traits. Different approaches are being carried out for this, amongst which molecular markers assisted breeding seems to be far reaching. Various molecular markers technology has been applied to cotton improvement program in general as well as to *G. arboreum* in specific. Continuous and intensive efforts are needed to create saturated maps as well as to identify of markers linked to fiber quality genes/QTLs. The improvement of *G. arboreum* fibers will be very fundamental to the gearing up of fiber industry as well as to the world economy.

### Conflict of Interests

The authors have not declared any conflict of interests.

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