Molecular Markers in Cotton Improvement

Ashok Kumar Meena¹*, N.V. Mohan Kumar¹, I.S. Katageri¹, Ramesh Methre¹ and Bheru Lal Kumhar²

¹University of Agricultural Sciences Dharwad, 580005, India
²Agricultural Research Station, Ummedganj Farm, Agriculture University, Kota, India

*Corresponding author

A B S T R A C T

Cotton is the very important commercial and cash crop, it is a very much necessary to improve the Superior agronomic traits to withstand against biotic and abiotic stress in the field and fiber qualities to meet requirement of advance spinning technology. Cotton improvement through conventional breeding is time consuming, in this context molecular markers found that efficient tool to accelerate the plant breeding program in cotton improvement. At present variety of molecular markers are available, choice of molecular marker depends on the user. This review article gives a over view of various molecular markers used in cotton include Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Sequence Related Amplified Polymorphism (SRAP) and Single Nucleotide Polymorphism (SNP). These markers play a crucial role in crop improvement program like (a) Analysis of Genetic diversity in cotton, (b) Construction of linkage map, (c) QTL analysis agronomic and fiber related traits in cotton (d) Marker assisted selection (MAS).

Keywords
RFLP, RAPD, AFLP, SSR, ISSR, SRAP, SNP, Genetic diversity, Linkage map, MAS

Introduction

Cotton (Gossypium spp) is the world’s most important natural and textile fiber crop, seeds are good source of vegetable oil and protein meal. Cotton is also called white gold because of economic importance, cotton alone contributes about 4% of national GDP. Cotton belongs to genus Gossypium family Malvaceae and genus Gossypium comprised of fifty species out of which forty five are diploid (2n=2x=26) and five are tetraploid (2n=4x=52), they occur in semiarid and arid areas of Africa, Central and South America, Galapagos, Indian subcontinent, Australia, Arabia, and Hawaii (Fryxell, 1992). The cultivated cotton includes, two diploid A genome species (G. arborium and G. herbacium) and two allotetraploid AD genome species (G. hirsutum and G. barbadense) (Wendel et al., 1999). In the world cotton is grown in an area of 33.1 million hectares producing 117 million bales with a productivity of 766 kg/ha (Dhruv, 2015). India ranks first in the world in terms of area under cotton cultivation 11.72 million
hectares and second in production with producing 372 lakh bales with a productivity of 541 kg/ha, Karnataka producing 15 lakh bales with a productivity of 464 kg/ha (Dhruv, 2015).

Plant breeders select the plants with desirable traits by looking at the phenotype. Most of these traits are polygenic in nature and many of them are influenced by environment. Although biometrical genetics provides the cumulative effects of the genetic loci involved in a polygenic trait but fails to identify locus involved in a particular trait. If the quantitative traits partition into individual genetic components by finding DNA marker closely linked to each trait, it would be easy to manipulate them efficiently and this would help to attain the desirable results quickly and more precisely (Preetha and Rveendren, 2008). These DNA markers will provide a information to the plant breeders to select desirable plants directly on the basis of genotype in the early stage itself, instead of waiting up to phenotype expression, where it is not possible through conventional breeding alone.

Generally aim of plant breeders is to improve Agronomically superior varieties or combining of interested traits present in different parental lines of cultivated species or their wild relatives. In order to combine all the favorable traits from different cultivars or related wild species for development of superior varieties through conventional breeding methods involve repeated backcrossing, selfing and testing which are time consuming and less precise processes as compared to direct selection of plants based on molecular processes (Preetha and Rveendren, 2008). Further conventional selection depends upon availability of lines with clear-cut phenotypic characters and accurate screening methods. The molecular marker techniques hasten the transfer of desirable genes from different varieties to background of single genotype and also introgress novel genes from related wild species into the local or popular genotypes, which would accelerate the generation of new varieties. In cotton there are different marker technologies are available ie., RFLPs, RAPDs, AFLPs, ISSRs, SSRs and SNPs, each marker have its own advantage and disadvantages. Tanksley (1983) listed five properties that distinguish molecular markers from morphological markers. These properties are (1) genotypes can be determined at the whole plant, tissue and/or cellular level (2) a relatively larger number of naturally occurring alleles exists at many loci, (3) phenotypic neutrality (4) alleles at many loci are codominant, (5) few epistatic or pleiotrophic effects are observed. In this brief background let us understand the role of particular marker in cotton improvement programme.

DNA marker techniques used in cotton

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism was the first kind of DNA marker. It belongs to hybridization based marker, which employs cloned DNA sequences to probe specific regions of the genome for variations that are seen as changes in the length of DNA fragments produced by digestion with restriction endonucleases (Landry et al., 1987). The main steps involve isolation of DNA, digestion with restriction enzymes (e.g., EcoRI, HindIII, DraI), separation of restricted fragments by agarose gel electrophoresis, transfer of fragments to nylon membrane, hybridization with probe and scoring of polymorphism by autoradiography.

RFLP was the first DNA marker used in crop improvement. Meredith (1992) in a study of
heterosis and varietal origins reported the first RFLP evaluation in upland cotton. Reinisch et al., (1994) developed first RFLP based linkage map of 4675 cM length with 41 linkage groups by using 705 RFLPs in cotton. Reinisch et al., (1994) reported that 46.2% of nuclear DNA probes detect RFLPs between Gossypium hirsutum and Gossypium barbadense, 64% are codominant in nature after that many scientists were used this markers in linkage map construction in cotton(Saranga et al., 2001; Paterson et al., 2003; Rong et al., 2004 and Chee et al., 2005). Yu et al., 1997 used RFLP markers for genetic diversity study in different cotton species. Wright et al., 1998, reported utility of RFLP markers in marker assisted selection (MAS) and RFLP linked to resistance allele for pathogen of bacterial blight was validated. RFLP markers are very complex and time and cost intensive technique which restricted it use, leading to development of less complicated techniques known as PCR base markers (Agarwal et al., 2008).

Random amplified polymorphic DNA (RAPD)

RAPD is the oldest PCR-based molecular marker technique it involves 10 bp random primer (Williams et al., 1990). It has many advantages over RFLP technique such as non-radioactive detection, it does not require prior sequence information, it required very small amount of genomic DNA, experimental simplicity and no need for expensive equipments beyond a thermocycler and a transilluminator (Rafalski, 1997). RAPD main disadvantage is that poor reproducibility (Jones et al., 1997). RAPD profile varies within and between laboratories because it is influenced by many factors like DNA concentration, reproducibility of thermocycler profiles, primer quality and concentration, choice of DNA polymerase, and pipetting accuracy (Rafalski, 1997).

RAPD techniques have been used for many purposes in cotton including assessment of, diversity, genome mapping, phylogenetic studies (Rahman et al., 2002; Zhang et al., 2002; He et al., 2008; Rahman et al., 2008; Rana and Bhat, 2004), genetic variations or diversity studies (Tatineni et al., 1996 Chalmers et al., 1992, Xu et al., 2001 and Chaudhary et al., 2010), DNA fingerprinting (Multani et al., 1995) and determining the relationship between the genotypes of different and same species (Wajahatullah et al., 1997), also used to evaluate the genetic relationship among cotton genotypes (Shu et al., 2001), to identify the QTLs for stomatal conductance (Ulloaand Meredith, 2000), to construct linkage mapping and QTL analysis in cotton (Zhang et al., 2003, Wang et al., 2006 and Lin et al., 2009). RAPDs were used to distinguish the cotton varieties resistant to jassids, aphids, and mites (Geng et al., 1995). RAPD marker (R-6592) for the male sterility gene has been identified in cotton (Lan et al., 1999).

Amplified fragment length polymorphism (AFLP)

AFLP technique was first developed by Vos et al., 1995, this technique combines reliability of RLFP with the ease of RAPD. The process involves three simple steps: (1) restriction of genomic DNA and ligation of oligonucleotide adaptors, (2) pre and selective amplification of restriction fragments and (3) gel analysis of amplified fragments. Generally polymorphic fragments are detected as present or absent making it a dominant marker system but in soybean Maughan et al., 1996 noticed codominant nature. The technique can be automated and allows the simultaneous analysis of many genetic loci per experiments. AFLP produces more polymorphic loci per primer than RFLPs, SSRs or RAPDs (Maughan et al., 1996).
AFLP is an effective tool for the observation of genetic diversity (Murtaza et al., 2006; Abdalla et al., 2001; Rana et al., 2005 and Li et al., 2008), fingerprinting studies, and tagging of agronomic, seed and fiberquality traits (Zhong et al., 2002; Rakshit et al., 2010 and Badigannavar et al., 2010). AFLP is a great valued technique for gene mapping studies due to their high abundance and random distribution throughout the genome (Vos et al., 1995). A linkage map of cotton was developed using the AFLP and RAPD markers (Altaf et al., 1997). AFLP markers have also been used for construction of linkage map and QTL analysis along with other markers (Yu et al., 2007; Wang et al., 2006; Lacape et al., 2009; Samer et al., 2015 and Cuming et al., 2015) and map saturation in cotton (Zhang et al., 2005 and Lacape et al., 2003).

**Inter Simple Sequence Repeats (ISSR)**

It allows the detection of polymorphism in inter SSR loci using primer (16–25 bp long) complimentary to a single SSR and anneal at either the 3’ or 5’ end (Khanam et al., 2012), that can be di, tri, tetra or pentanucleotide (Reddy et al., 2002). The technique of ISSR markers combines many benefits of AFLPs and SSRs with universality of RAPDs (Bornet et al., 2001). Generally the sequence of ISSR primers is larger as compare to RAPD primers, allowing higher annealing temperature which outcomes greater reproducibility of bands than RAPDs (Reddy et al., 2002, Culley et al., 2000). Amplification of ISSRs also revealed larger fragments number per primer than RAPDs (Wang and Yi, 2002). Many earlier studies reported that ISSR markers were more informative than RAPDs for genetic diversity evaluation in different crop species (Nagaoka et al., 1997; Galván et al., 2003). The applications of ISSRs for different purposes depend on the diversity and frequencies of SSR within the particular genomes (Shi et al., 2010). It is quickly being utilized by the research community in different areas of plant improvement, that is, in gene tagging, analysis of genetic diversity, and estimation of SSR motif (Blair et al., 1999; Bornet et al., 2002 and Sica et al., 2005). ISSRs have been reported as quite useful markers for revealing polymorphism in cotton genotypes (Liu et al., 2001).

**Simple Sequence Repeats (SSR)**

These are di-, tri-, tetra- or pentatandom repeats of nucleotide, scattered abundantly in both noncoding and coding regionsof a genome (Kalía et al., 2011; Khanam et al., 2012). Microsatellites are created from sphere where variants of repetitive DNA sequence are previously overrepresented (Tautz et al., 1986). The loci of these markers are highly transferable about 50% across species (Saha and Jenkins, 2004). For SSRs analysis forward and reverse primers are employed in PCR reaction that anneal to the template DNA at the 5’ and 3’ ends. Short repetitive DNA sequences furnish the basis for multi allelic, co dominant PCR based molecular marker and found more polymorphic as compare to other DNA markers (Preetha and Raveendren, 2008; Khanam et al., 2012). Due to their greater polymorphism, SSRs are considered as an important marker system in fingerprinting, analysis of genetic diversity (Qayyum et al., 2009 and Arunita et al., 2010), molecular mapping and marker assisted selection (Reddy et al., 2001). According to Blenda et al., (2006), there are various uses of microsatellites for plant breeders such as selective breeding improvement, genetic diversity estimation, introducing novel genes into breeding materials from exotic germplasm, cultivar protection, locating qualitative and quantitative trait loci.
Several methods have been pursued to develop SSR markers in cottons, including analysis of SSR-enriched small insert genomic DNA libraries (Kalia et al., 2011; Udall et al., 2006; Ince et al., 2010; Richard et al., 1995), SSR mining from ESTs (Shaheen et al., 2009), and large-insert BAC derivation by end sequence analysis (Reddy et al., 2002). Cotton researchers have explored simple sequence repeats (SSRs) for studies the phylogenetic and diversity analysis (He et al., 2007; Lacape et al., 2007) genetic mapping and QTL analysis for different traits (Park et al., 2005; Xiao et al., 2009; Yu et al., 2012, Michael et al., 2014 and Tang, et al., 2015), association mapping (Kantartzi and stewart, 2008).

**Sequence related amplified polymorphism (SRAP)**

SRAP marker technique was introduced by Li and Quiros (2001), this new marker technique preferentially amplifies ORFs through PCR by using two different primer pair. First one forward primer, which contains 17 base pairs, in that 14 nucleotide sequence rich in G and C in the 5’end and three selective bases in the 3’end. This primer amplifies preferentially exonic regions. The second one is reverse primer with 19 base pairs, contains a sequence of 16 nucleotide rich in A and T in the 5’end and three selective bases in the 3’end. This primer preferentially amplifies intronic regions and regions with promoters. This technique combines simplicity, reliability, moderate throughput ratio and facile sequencing of selected bands. Further, it targets coding sequences in the genome and results in a moderate number of co dominant markers. However, these techniques will not utilize any prior sequence information, and the markers generated are randomly distributed across the genome. In cotton this new marker technique is being used along with other markers (Lin et al., 2005; He et al., 2007; Lin et al., 2009; Zhang et al., 2009 and Yu et al., 2007) for saturating the genome.

**Single Nucleotide Polymorphism (SNP)**

Variations of single nucleotide (A, T, C, G) in sequence of individual genome are known as single nucleotide polymorphism or SNPs (Agarwal et al., 2008). These may occur in the non coding, coding and intergenic regions of the genome, so allowing the detection of the genes due to the variations in the sequences of nucleotides (Agarwal et al., 2008) and these are either non synonymous or synonymous within the coding regions of the genome. Synonymous changes can alter mRNA splicing that result the changes in the phenotype of an individual (Richard et al., 1995). The main advantage of SNP markers is to relate their ease of data management along with their flexibility, speed and cost-effectiveness. Bi-allelic SNP markers are straight forward to merge data across groups and create large databases of marker information, since there are only two alleles per locus and different genotyping platforms will provide the same allele calls once proper data has been performed.

SNP markers are important tool for linkage mapping, map based cloning and marker assisted selection due to the high level of polymorphism. The co dominant nature of SNPs makes these markers able to distinguish the heterozygous and homozygous alleles (Shaheen et al., 2009). Because of high polymorphism nature SNPs were used to observe diversity, characterization, mapping and for construction of linkage map and QTL analysis in cotton (Michael et al., 2014 and Hulse-Kemp et al., 2015).

Recently, an international collaborative effort has developed 70K SNP chip based on Illumina Infinium genotyping assay
(Unpublished data; http://www.cottongen.org/node/1287616). This high-throughput genotyping assay will be a resource that will be used globally by public and private breeders, geneticists, and other researchers to enhance cotton genetic analysis, breeding, genome sequence assembly, and many other uses.

Important applications of molecular markers in cotton improvement

Genetic diversity studies in cotton

Success of breeding program depends on the understanding of genetic diversity within and among genetic resources of the available germplasm and enable plant breeders to choose parental sources that will generate diverse populations for selection. Characterization of genetic similarity among genotypes is a valuable source to select parental combinations for maintaining genetic diversity in a breeding program (Beceelaere et al., 2005). The knowledge of genetic relationships among plant genotypes helps to know the complexity available germplasm, to discover the differences in available genotypes and to build up useful conservation plans (Dahab et al., 2013). Thus, evaluation based on the molecular markers can give valuable insight into the genetic structure of a plant population, which helps in the development of new varieties. There are many genetic diversity studies have been carried out in cotton by employing different marker techniques e.g. amplified fragment length polymorphism (AFLP) (Abdalla et al., 2001; Rana et al., 2005; Li et al., 2008), random amplified polymorphic DNA (RAPD) (Xu et al., 2001; Chaudhary et al., 2010) and simple sequences repeats (Qayyum et al., 2009; Arunita et al., 2010). A overview of some published genetic diversity studies by using molecular markers is depicted in Table 1.

Genetic linkage map construction in cotton

Genetic mapping (also known as linkage mapping or meiotic mapping) refers to the determination of the relative position and distances between markers along chromosomes. Genetic map distances between two markers are defined as the mean number of recombination events, involving a given chromatid, in that region per meiosis. Genetic linkage maps are fundamental for the localization of genes conferring biotic and abiotic stress tolerance. Genetic maps based on molecular markers have several advantages over classical maps. Genetic mapping can be developed by different mapping populations, but popularly F2, backcross and recombinant inbred lines these three populations were used for construction of genetic linkage map in plants (Paterson, 1996). Molecular map of the cotton genome was first constructed using 705 RFLP loci and partitioned into 41 linkage groups (Reinisch et al., 1994). Many more cotton molecular maps have been developed and published. An overview of published genetic linkage maps in cotton is given in Table 2.

QTL mapping for yield, yield contributing and fiber quality trait in cotton

The regions in genomes to have genes linked with a quantitative trait are known as quantitative trait loci, QTLs (Collard et al., 2005), and the process of developing linkage maps and performing QTL analysis is referred to as QTL mapping (Paterson et al., 1996 and Paterson et al., 1996). QTL analysis stands on the principal of identifying a connection among phenotype and genotype of markers. The QTLs identified in cotton using different marker technologies are listed in Table 3. These identified QTLs are the new avenue to accelerate the cotton improvement through marker assisted selection.
Marker-Assisted Selection (MAS)

Marker assisted selection (MAS) is a procedure by which a phenotype is selected on the basis of genotype of a marker (Collard et al., 2005). Once the markers tightly linked to the genes have been detected, breeders may use particular DNA marker to identify the plants carry the genes (Young et al., 1996). The effectiveness and cost of MAS are influenced by the marker technique; therefore, it must be selected carefully (Young et al., 1996). During the past two decades, RAPDs techniques have been used for MAS for getting the ginned plants and ginnedless seeds in the interspecific population of G. sturtianum and other species. It was exposed that DNA markers connected to the major QTL (QTLFS1) for fiber strength could be utilized in MAS to increase fiber strength of commercial varieties in segregating populations (Zhang et al., 2003). SSR markers namely CIR 316 tightly linked to Root knot nematod (RKN) resistant region on chromosome 11 and BNL 3661 marker tightly linked to RKN resistant region on chromosome 14.

Jenkins et al., (2012) by using these SSR markers selected 11 homozygous plants for chromosome 11 and 14 from F2 population derived from RKN resistant genotype M 240 RNR ×susceptible cultivar FM966 instead of waiting up to F6-F8 through conventional breeding. That selected plant confirmed resistance against the RKN. In cotton it is necessary to identify specific genes for particular traits like fiber length, strength, fineness...etc, to combine these genes from different genotypes through marker assisted selection. Some of identified genes for particular traits is depicted in Table 4.

| S. No. | Country | Population type | Markers used | References |
|-------|---------|-----------------|--------------|------------|
| 1     | India   | 150 G. hirsutum lines | 50 SSR       | Rajeev et al., 2014 |
| 2     | India   | Intraspecific cotton F1 hybrids and its parents | 20 RAPD and 19 ISSR | Dongre et al., 2012 |
| 3     | USA     | 24 lines of cotton | 270 SNP loci and 92 Indel | Van et al., 2009 |
| 4     | India   | 24 lines of G. hirsutumL. | 6 AFLP primers | Rana et al., 2005 |
| 5     | USA     | 24 cultivars of G. hirsutum | 88 SSR primers | Zhang et al., 2005 |
| 6     | Pakistan | 31 Gossypium species, 3 subspecies and 1 interspecific hybrid | 45 RAPD primers | Khan et al., 2000 |

Table 1 An overview of genetic diversity studies in cotton by using molecular markers
Table 2a Overview of published genetic linkage maps in cotton

| S. No. | Interspecific crosses | Types | SIZE | Mapping population | Markers | No. of mapped loci | Map Length (cM) | No.of LGs | Reference |
|--------|-----------------------|-------|------|-------------------|---------|-------------------|-----------------|----------|-----------|
| 1      | *Gh* (palmeri) × *Gb* (K101) | F₂ | 57 | RFLP | 705 | 4675 | 41 | Reinisch et al., 1994 |
| 2      | *Gh* (CAMD-E) × *Gb* (Sea Island Seaberry) | F₂ | 271 | RFLP | 261 | 3767 | 27 | Jiang et al., 1998 |
| 3      | *Gh* (Delta pine 61) × *Gb* (Sea Island Seaberry) | F₂ | 180 | RFLP | - | 3664 | 26 | Jiang et al., 2000 |
| 4      | *Gh* (Siv'on) × *Gb* (F-177) | F₂ | 171 | RFLP, RAPD and SSR | - | 4766 | 50 | Kohel et al., 2001 |
| 5      | *Gh* (Siv'on) × *Gb* (F-177) | F₂ | 430 | RFLP | 253 | - | - | Saranga et al., 2001 |
| 6      | *Gh* (Siv'on) × *Gb* (F-177) | F₂ | 208 | RFLP | - | - | - | Paterson et al., 2003 |
| 7      | *Gh* (TM 1) × *Gb* (Hai7124) × TM1 | BC₂/F₁ | 140 | EST-SSR | 624 | 5644.3 | 54 | Han et al., 2004, 2006 |
| 8      | *Gh* (Pima 44) × *Gb* (Pima S7) | F₂ | 94 | AFLP, SSR, and RFLP | 392 | 3287 | 42 | Mei et al., 2004 |
| 9      | *Gh* (Palmeri) × *Gb* (K101) | F₂ | 57 | RFLP | 2584 | 4447.9 | 26 | Rong et al., 2004 |
| 10     | *Gh* (Tamcot 2111) × *Gb* (Pima S6) × Tamcot 2111 | BC₂/F₂ | 3662 | RFLP | - | - | - | Chee et al., 2005 |
| 11     | *Gh* (Guzuncho 2) × *Gb* (VH8) × Guazuncho 2 | BC₁ and BC₂ | 200 | SSR and RFLP | 1306 | 5597 | 26 | Lacape et al., 2003, Lacape et al., 2005 |
| 12     | *Gh* (Handan 2008) × *Gb* (Pima90) | F₂ and F₂,3 | 69 | SSR, SRAP, RAPD, and REMAPS | 1029 | 5472.3 | 26 | Lin et al., 2005, He et al., 2007 |
| 13     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 183 | EST-SSR | 193 | 1277 | 19 + 11 LG | Park et al., 2005 |
| 14     | *Gh* (TM 1) × *Gb* (Pima 3-79) | F₂ and F₂,1 | 163 | SSR | 86 | 666.7 | 21 | Shen et al., 2005 |
| 15     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 183 | SSR | 433 | 2126.3 | 46 | Frelíchková et al., 2006 |
| 16     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 186 | SSR, TRAP, SRAP, and AFLP | 1097 | 4536.7 | 35 | Yu et al., 2007 |
| 17     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 121 | SSR | - | 5472.3 | 26 | He et al., 2008 |
| 18     | *Gh* (Guzuncho 2) × *Gb* (VH8-4602) | RILs | 140 | SSR and AFLP | 800 | 2044 | 26 | Lacape et al., 2009 |
| 19     | *Gh* (Guzuncho 2) × *Gb* (VH8-4602) | RILs | 62 | SSR | 57 | 911.6 | 19 | Santoshkumar et al., 2010 |
| 20     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 186 | SSR and SRAP | 2072 | 3380 | 26 | Yu et al., 2012 |
| 21     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 146 | SSR | 392 | 2,895 | 26 | Yu et al., 2013 |
| 22     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 98 | SSR and SNP | 841 | 2061 | 26 | Michael et al., 2014 |
| 23     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 118 | SNP | 19,198 | 4,439.6 | 0.23 | Hulse-Kemp et al., 2015 |
| 24     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 60 | AFLP, SSR, EST-SSR | 210 | 3503.8 | 26 | Samer et al., 2015 |
| 25     | *Gh* (TM 1) × *Gb* (Pima 3-79) | RILs | 82 | SSR | 589 | 4259.4 | 52 | Westengen et al., 2005 |
| Intraspecific crosses | Type | Size | Markers | No. of mapped loci | Map Length (cM) | No. of LGs | Reference |
|-----------------------|------|------|---------|------------------|----------------|------------|-----------|
| 1. Gh(HS46) × Gh(MARCABUCAG8US-1-88) F2 and F3 | F2 and F3 | 96 | RFLP | 120 | 865 | 31 | Shappley et al., 1998 |
| 2. Gh × Gh | F2 | 569 | RFLP | 284 | 1502.6 | 47 | Ulloa et al., 2002 |
| 3. Gh(TM 1) × G. anomalum(7235) | F2 and F3 | 186 | SSR and RAPD | - | - | - | Zhang et al., 2003 |
| 4. Gh (Handan208) × Gh(Pima90) | F2 | 129 | SRAP | 237 | 3030.7 | 39 | Lin et al., 2005 |
| 5. Gh (Acala 44) × Gh(Pima S7) | F2 | 94 | AFLP, SSR, and RFLP | 392 | 3287 | 42 | Mei et al., 2004 |
| 6. G. trilobum(Skovsted) × G. raimondii(Ulbr) | F2 | 62 | RFLP | 763 | 1493.3 | 13 | Rong et al., 2004 |
| 7. Gh(Yumian 1) × Gh(T586) | F2 and F2,3 | 117 | SSR and AFLP | 70 | 525 | 20 | Zhang et al., 2005 |
| 8. Gh(TM1) × Gh(7235) | RILs | 258 | SSR | 110 | 810.07 | 22 | Shen et al., 2007 |
| 9. Gh(Zhongmiansuo12) × Gh(8891) | RILs | 180 | SSR, AFLP, RAPD, and SRAP | 132 | 865.20 | 26 | Wang et al., 2006 |
| 10. Gh(L-70) × Gh(L-47) | RILs | 76 | EST-SSR | - | - | - | Abdurakhmonov et al., 2007 |
| 11. Gh(7235) × Gh(TM-1) | RILs | 207 | SSR | 156 | 1024.4 | 31 | Shen et al., 2007 |
| 12. Gh(Yumian 1) × Gh(T586) | RILs | 270 | SSR | 19 | 96.2 | 1 | Wan et al., 2007 |
| 13. Gh(Deltapine) × Gh(Texas 701) | F2 | 251 | SSR | 73 | 650.8 | 17 | Guo et al., 2008 |
| 14. Gh × Gh | 4WC | 273 | SSR, EST-SSR | 286 | 2113.3 | 56 | Qin et al., 2008 |
| 15. Gh(DH962) × Gh(Jimian5) | F2 | 137 | SRAP, SSR, RAPD and RGAP | 471 | 3070.2 | 51 | Lin et al., 2009 |
| 16. Gh (HS 46) × Gh (MARCABUCAG8US-1-88) | RILs | 188 | SSR | 125 | 965 | 26 | Wu et al., 2009 |
| 17. Gh(Yumian 1) × Gh(T586) | RILs | 270 | SSR and SRAP | 604 | 3140.9 | 60 | Zhang et al., 2009 |
| 18. Gh(Yumian 1) × Gh(T586) | F2 | 124 | SSR, EST-SSR, SNP | 412 | 2108.34 | 52 | Wang et al., 2013 |
| 19. Gh (Yumian 1 × T723) | RILs | 180 | SSR | 1,540 | 2,842.06 | 26 | Tang et al., 2015 |
| 20. Gh (Yesil × Nazilli 84) | F2 | 94 | AFLP | 240 | 2068.5 | 27 | Cumming et al., 2015 |
### Table 3 List of QTLs identified in cotton

| Sl.No. | Traits                  | Descriptor                        | Population | Marker (number and Type) | QTLs No. | Reference          |
|-------|-------------------------|-----------------------------------|------------|--------------------------|----------|--------------------|
|       |                         |                                   |            |                          |          |                    |
| 1     | Fiber quality           | FS, FL, FF                        | F₂         | 216 RFLP, 139 RAPDs      | 13       | Kohel et al., 2001 |
|       |                         | FS                                | F₂         | 217 SSRs, 800 RAPDs UBC and 1040 OPERON | 2        | Zhang et al., 2003 |
|       |                         | LY, LP, SW, NS, UQ, SF, FL, FE, FF and IF | F₂         | 144 AFLPs, RFLPs and 150 SSRs | 28       | Mei et al., 2004  |
|       |                         | FS, FE, FF, FU and FL             | F₂         | 448 AFLPs                | 28       | Zhang et al., 2011 |
|       |                         | FS, FE, FL, FU, LP and FF         | F₂         | 290 SSRs and 9 AFLPs     | 16       | Zhang et al., 2003 |
|       |                         | FF                                | F₂         | 262 AFLPs                | 41       | Draye et al., 2005 |
|       |                         | FL, FLU and SFC                   | BC3F₂      | 262 RFLPs                | 45       | Chee et al., 2005  |
|       |                         | FS, FL, FF, FE                    | RIL’s      | 95 SSRs, 72 CSR          | 13       | Park et al., 2005  |
|       |                         | FL, FS, FF and FE                 | F₂         | 1378 SSRs                | 39       | Shen et al., 2005  |
|       |                         | FS, FL, FF, FMT, FE and SFI       | RIL’s      | 4106 SSRs, AFLPs, RAPDs and SRAPs | 48       | Wang et al., 2006  |
|       |                         | FE, FL, FU, FL and FF             | RIL’s      | 7508 SSRs, 384 SRAPs and 740 IT-ISJs | 13       | Zhang et al., 2009 |
|       |                         | FE, FS, FF and FU                 | CP         | 16052 SSRs               | 63       | Zhang et al., 2012 |
|       |                         | FE, FL, FF and FU                 | RIL’s      | 25,313 SSRs              | 62       | Tang et al., 2015  |
|       | Fiber and agronomical   | SCY, LY, LP, BW, SI, FMT, PER, WF, WT, FF, FL, FE and FS | F₂         | 123 AFLPs                | 43       | Cuming et al., 2015 |
|       | Yield and fiber         | BW, LP, FF, ES, FU, DFF and DFN   | F₂         | 50 EST, 18 EST-SSR, 36 SSRs and 64 AFLP | 81       | Samer et al., 2015 |
|       |                         | SCY, LI, SI, LY, no. of seeds per boll, FS, FL and FF | RIL’s      | 141 SSRs                 | 36       | Wu et al., 2009    |
|       |                         | SCY, LY, LP, SI, NB, SCY and LY   | RIL’s      | 834 SSRs, 437 SRAPs, 107 RAPDs, 16 REMAPs | 57       | He et al., 2008    |
|       |                         | FS, FL, FF, FE, LP, SI, NB, SCY and LY | RIL’s      | 2131 SSRs                | 53       | Shen et al., 2007  |
|       |                         | LI, SI, LY, SCY, NSB and FS       | F₂         | 834 SSRs, 437 SRAPs, 107 RAPDs and 16 REMAPs | 52       | He et al., 2007    |
|       |                         | NB, BW, SI, LP, LI, SCY, LY, FL, FS, FF and FU | 4WC and inbred lines | 6123 SSRs and EST-SSRs | 31       | Qin et al., 2008   |
|       |                         | SCY, LY, NB, BW, LP, SI, LI and FBN | RIL’s and IF2 | 2675 EST-SSRs          | 111      | Liu et al., 2012   |
|       |                         | PH, FBN, BW, LP, LI, SI, LY, FL, FS, FF and FU | G. hirsutum accessions | 121 SSRs               | 180      | Zhang et al., 2013 |
|       |                         | SCY, LY,LI, BW, FL, FS, FU        | BILs       | 2,041 SSRs               | 67       | Yu et al., 2013    |

NB: number of bolls per plant, BW: boll weight, SI: seed index, LP: lint percent, LI: lint index, SI: seed index, SCY: seed cotton yield per plant, LY: lint yield per plant, FL: fiber length, FS: fiber strength, FE: fiber elongation, FU: fiber uniformity ratio, FY: fiber yellowness, FF: fiber fineness, FMT: fiber maturity, PH: plant height, FBL: fruit branch length, FBN: fruit branch number, FBA: fruit branch angle, FLU: fiber length uniformity, SFC: short fiber content, FR: fiber reflectance, SW: seed weight, NS: number of seeds per bolls, UQ: upper quartile length, SF: short fiber content, FT: fiber tenacity, IF: immature fiber content, SFI: short fiber index, NSB: number of seeds per boll, Date of 1st Flowering (DFF), Node of 1st Fruiting Branch (FFN).
Table 4 Over view of specific genes identified in cotton for particular traits

| Traits                        | Genes          | Reference                                      |
|-------------------------------|----------------|-----------------------------------------------|
| Fiber strength                | qFs1           | Zhang et al. 2003                             |
| Fiber length                  | qFL-D2-1       | Wang et al., 2006                             |
| CMS                           | Rf1, Rf2       | Lan et al. 1999 Liu et al. 2003, Zhang et al. 2005 |
| GMS                           | ms1, ms6, ms15 | Chen et al., 2009                             |
| Fiber development             | Li1, Li2, N1, N2, Fz, ha N1, n1, n2 | Rong et al., 2007                             |
| Leaf shape                    | L3, P1, Y1, t1, T1 | Song et al., 2005; Guo et al., 2006; Wright et al., 1999 |
| Glandless                     | gl1, gl2, gl3, gl4, gl5, and gl6 | Pauly, 1979                                    |
| Gladness                      | G11 and G12    | Lee, 1965; McCarty et al., 1996               |
| Root-knot nematode resistance | rkn1           | Wang et al., 2006, Shen et al., 2006           |
| Blight resistance             | B2, B3, B12    | Wright et al., 1998                           |
| Fusarium resistance           | FWR            | Wang et al., 2009                             |

In conclusion, marker mediated varietal fingerprinting and germplasm characterization Molecular Marker-Assisted Technologies for cotton Improvement appeared most common and most pervasive application with AFLP and SSR markers. Being cost effective, easy to handle and devoid of any radioisotope requirement, SSR and SNP markers are considered as the most suitable and reliable system for DNA fingerprinting. Marker-assisted selection has been successful for introgressing and pyramiding major-effect genes, however many challenges remain to be resolved before MAS can routinely provide added value for breeding very complex traits. Marker-assisted selections for qualitative traits appeared most successful after DNA fingerprinting while for quantitative characters, insect resistance genes and genes controlling QTL for abiotic stress tolerance, the success is limited. It is anticipated that application of markers will remain restricted in these areas till the allele-specific markers are available and the cost of marker analysis is reduced significantly. Although there have been numerous QTL mapping studies for a wide range of traits in cotton crop, relatively few markers have actually been implemented in breeding programs for cotton improvement. The rate, scale, and scope of uptake of MAS in public crop breeding program has continually lagged behind expectations. There are many technical and logistical factors that have hindered the speed and scope of MAS uptake. Steady progress and advancement in DNA markers will make it more attractive for molecular breeding and plant genetics and ultimately help in cotton improvement.

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