

Review Article

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## Molecular Markers in Cotton Improvement

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### ABSTRACT

#### Keywords

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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).

### 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 its use, leading to development of less complicated techniques known as PCR based 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 (Ulloa and 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 RFLP 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 fiber quality 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; Galv'an *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 pentanucleotide repeats of nucleotide, scattered abundantly in both noncoding and coding regions of a genome (Kalia *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 (Guo *et al.*, 2007; Lacape *et al.*, 2009; 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 (Becelaere *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 F<sub>2</sub>, 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 glanded plants and glandless 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  $\times$  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.

**Table.1** An overview of genetic diversity studies in cotton by using molecular markers

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

**Table.2a** Overview of published genetic linkage maps in cotton

S. No.	Cross parents	Mapping population		Markers	No. of mapped loci	Map Length (cM)	No.of LGs	Reference
		Types	SIZE					
<b>Interspecific crosses</b>								
1.	<i>Gh</i> (palmeri) × <i>Gb</i> (K101)	F <sub>2</sub>	57	RFLP	705	4675	41	Reinischet.al., 1994
2.	<i>Gh</i> (CAMD-E) × <i>Gb</i> (Sea Island Seaberry)	F <sub>2</sub>	271	RFLP	261	3767	27	Jiang et.al., 1998
3.	<i>Gh</i> (Deltapine 61) × <i>Gb</i> (Sea IslandSeaberry)	F <sub>2</sub>	180	RFLP	-	3664	26	Jiang et.al., 2000
4.	<i>Gh</i> (TM1) × <i>Gb</i> (3-79)	F <sub>2</sub>	171	RFLP, RAPD and SSR	-	4766	50	Kohel et.al., 2001
5.	<i>Gh</i> (Siv'on) × <i>Gb</i> (F-177)	F <sub>2</sub>	430	RFLP	253	-	-	Saranga et.al., 2001
6.	<i>Gh</i> (Siv'on) × <i>Gb</i> (F-177)	F <sub>3</sub>	208	RFLP	-	-	-	Paterson et.al.,2003
7	( <i>Gh</i> (TM 1) × <i>Gb</i> (Hai7124)) × TM1	BC <sub>1</sub> F <sub>1</sub>	140	EST-SSR	624	5644.3	34	Han et.al.,2004,2006
8	<i>Gh</i> (Acala 44) × <i>Gb</i> (Pima S7)	F <sub>2</sub>	94	AFLP, SSR, and RFLP	392	3287	42	Mei et.al., 2004
9	<i>Gh</i> (Palmeri) × <i>Gb</i> (K101)	F <sub>2</sub>	57	RFLP	2584	4447.9	26	Rong et.al., 2004
10	( <i>Gh</i> (Tamcot 2111) × <i>Gb</i> (Pima S6))×Tamcot 2111	BC <sub>3</sub> F <sub>2</sub>	3662	RFLP	-	-	-	Chee et.al., 2005
12	( <i>Gh</i> (Guazuncho 2) × <i>Gb</i> (VH8)) × Guazuncho 2	BC <sub>1</sub> and BC <sub>2</sub>	200	SSR and RFLP	1306	5597	26	Lacape et.al.,2003, Lacape et.al., 2005
13	<i>Gh</i> (Handan208) × <i>Gb</i> (Pima90)	F <sub>2</sub> and F <sub>2,3</sub>	69	SSR, SRAP, RAPD, and REMAPs	1029	5472.3	26	Lin et.al., 2005, He et.al., 2007
14	<i>Gh</i> (TM1) × <i>Gb</i> (Pima 3-79)	RILs	183	EST-SSR	193	1277	19 + 11 LG	Park et.al., 2005
15	<i>Gh</i> (7235) × <i>Gb</i> (TM-1)	F <sub>2</sub> and F <sub>2,3</sub>	163	SSR	86	666.7	21	Shen et.al., 2005
16	<i>Gh</i> (TM1) × <i>Gb</i> (3-79)	RILs	183	SSR	433	2126.3	46	Frelichowski et.al., 2006
17	<i>Gh</i> (CRI36) × <i>Gb</i> (Hai7124)	F <sub>2</sub>	186	SSR, TRAP, SRAP, and AFLP	1097	4536.7	35	Yu et.al., 2007
18	<i>Gh</i> (Handan 208) × <i>Gb</i> (Pima 90)	RILs	121	SSR	-	5472.3	26	He et.al., 2008
19	<i>Gh</i> (Guazuncho 2) × <i>Gb</i> (VH8-4602)	RILs	140	SSR and AFLP	800	2044	26	Lacape et.al., 2009
20	( <i>Gh</i> (KC3) × <i>Gb</i> (Suvin)) × KC3	BC <sub>1</sub> F <sub>1</sub>	62	SSR	57	911.6	19	Santoshkumarr et.al., 2010
21	<i>Gh</i> (TM1) × <i>Gb</i> (3-79)	RILs	186	SSR and SNP	2072	3380	26	Yu et al., 2012
22	<i>Gh</i> (SG 747) × <i>Gb</i> (Giza 75)	BILs	146	SSR	392	2,895	26	Yu et al., 2013
23	<i>Gh</i> (TM-1) × <i>Gb</i> (NM24016)	RILs	98	SSR and SNP	841	2061	26	Michael et al., 2014
24	<i>Gb</i> .doubled haploid line 3- 79 x <i>G. hirsutum</i> cv. Texas Marker- 1	F <sub>2</sub>	118	SNP	19,198	4,439.6	0.23	Hulse-Kemp et al., 2015
25	Giza 45 ( <i>G. barbadense</i> ) x Tamcot Luxor ( <i>G. hirsutum</i> )	F <sub>2</sub>	60	AFLP, SSR, EST-SSR	210	3503.8	26	Samer et al., 2015
26	<i>Gh</i> (TMS22) × <i>G.tomentosum</i> (WT936)	F <sub>2</sub>	82	SSR	589	4259.4	52	Westengen et al., 2005



**Table.2b** Overview of published genetic linkage maps in cotton

	Intraspecific crosses	Type	Size	Markers	No. of mapped loci	Map Length (cM)	No.of LGs	Reference
1	<i>Gh</i> (HS46) × <i>Gh</i> (MARCABUCAG8US-1-88) F2 and F3 96 RFLP 120 865 31	F <sub>2</sub> and F <sub>3</sub>	96	RFLP	120	865	31	Shappley <i>et al.</i> , 1998
2	<i>Gh</i> × <i>Gh</i>	F <sub>2:3</sub>	569	RFLP	284	1502.6	47	Ulloa <i>et al.</i> , 2002
3	<i>Gh</i> (TM 1) × <i>G.anomalum</i> (7235)	F <sub>2</sub> and F <sub>3</sub>	186	SSR and RAPD	-	-	-	Zhang <i>et al.</i> , 2003
4	<i>Gh</i> (Handan208) × <i>Gh</i> (Pima90)	F <sub>2</sub>	129	SRAP	237	3030.7	39	Lin <i>et al.</i> , 2005
5	<i>Gh</i> (Acala 44) × <i>Gb</i> (Pima S7)	F <sub>2</sub>	94	AFLP, SSR, and RFLP	392	3287	42	Mei <i>et al.</i> , 2004
6	<i>G. trilobum</i> (Skovsted) × <i>G. raimondii</i> (Ulbr)	F <sub>2</sub>	62	RFLP	763	1493.3	13	Rong <i>et al.</i> , 2004
7	<i>Gh</i> (Yumian 1) × <i>Gh</i> (T586)	F <sub>2</sub> and F <sub>2:3</sub>	117	SSR and AFLP	70	525	20	Zhang <i>et al.</i> , 2005
8	<i>Gh</i> (TM1) × <i>Gh</i> (7235)	RILs	258	SSR	110	810.07	22	Shen <i>et al.</i> , 2007
9	<i>Gh</i> (Zhongmiansuo12) × <i>Gh</i> (8891)	RILs	180	SSR, AFLP, RAPD, and SRAP	132	865.20	26	Wang <i>et al.</i> , 2006
10	<i>Gh</i> (L-70) × <i>Gh</i> (L-47)	RILs	76	EST-SSR	-	-	-	Abdurakhmonov <i>et al.</i> , 2007
11	<i>Gh</i> (7235) × <i>Gh</i> (TM-1)	RILs	207	SSR	156	1024.4	31	Shen <i>et al.</i> , 2007
12	<i>Gh</i> (Yumian 1) × <i>Gh</i> (T586)	RILs	270	SSR	19	96.2	1	Wan <i>et al.</i> , 2007
13	<i>Gh</i> (Deltapine) × <i>Gh</i> (Texas 701)	F <sub>2</sub>	251	SSR	73	650.8	17	Guo <i>et al.</i> , 2008
14	<i>Gh</i> × <i>Gh</i>	4WC	273	SSR, EST-SSR	286	2113.3	56	Qin <i>et al.</i> , 2008
15	<i>Gh</i> (DH962) × <i>Gh</i> (Jimian5)	F <sub>2</sub>	137	SRAP, SSR, RAPD and RGAP	471	3070.2	51	Lin <i>et al.</i> , 2009
16	<i>Gh</i> (HS 46) × <i>Gh</i> (MARCABUCAG8US-1-88)	RILs	188	SSR	125	965	26	Wu <i>et al.</i> , 2009
17	<i>Gh</i> (Yumian 1) × <i>Gh</i> (T586)	RILs	270	SSR and SRAP	604	3140.9	60	Zhang <i>et al.</i> , 2009
18	<i>Gb</i> (Hai7124 × <i>Gb</i> (3-79)	F <sub>2</sub>	124	SSR, EST-SSR, SNP	412	2108.34	52	Wang <i>et al.</i> , 2013
19	<i>Gh</i> (Yumian 1 × 7235)	RILs	180	SSR	1,540	2,842.06	26	Tang <i>et al.</i> , 2015
20	<i>Gh</i> (Yesil × Nazilli 84)	F <sub>2</sub>	94	AFLP	240	2068.5	27	Cuming <i>et al.</i> , 2015

**Table.3** List of QTLs identified in cotton

Sl.No.	Traits	Descriptor	Population		Marker (number and Type)	QTLs No.	Reference
			Type	Size			
1	Fiber quality	FS, FL, FF	F <sub>2</sub>	171	216 RFLP, 139 RAPDs	13	Kohel <i>et al.</i> , 2001
		FS	F <sub>2</sub>	186	217 SSRs, 800 RAPDs UBC and 1040 OPERON	2	Zhang <i>et al.</i> , 2003
		LY, LP, SW, NS, UQ, SF, FL, FE, FT, FF and IF	F <sub>2</sub>	120	144 AFLPs, RFLPs and 150 SSRs	28	Mei <i>et al.</i> , 2004
		FS,FE, FF, FU and FL	F <sub>2</sub>	200	448 RFLP	28	Zhang <i>et al.</i> , 2011
		FS, FE, FL, FU, LP and FF	F <sub>2</sub>	117	290 SSRs and 9 AFLPs	16	Zhang <i>et al.</i> , 2005
		FF	BC3F <sub>2</sub>	3,662	262 RFLPs	41	Draye <i>et al.</i> , 2005
		FL, FLU and SFC	BC3F <sub>2</sub>	3,662	262 RFLPs	45	Chee <i>et al.</i> , 2005
		FS, FL, FF, FE	RILs	---	95 SSRs, 72 CSR	13	Park <i>et al.</i> , 2005
		FL, FS, FF and FE	F <sub>2</sub>	---	1378 SSRs	39	Shen <i>et al.</i> , 2005
		FS, FL, FF, FMT, FE and SFI	RIL's	180	4106 SSRs, AFLPs, RAPDs and SRAPs	48	Wang <i>et al.</i> , 2006
		FS, FE, FU, FL and FF	RIL's	270	7508 SSRs, 384 SRAPs and 740 IT-ISJs	13	Zhang <i>et al.</i> , 2009
		FE, FL, FS, FF and FU	CP	172	16052 SSRs	63	Zhang <i>et al.</i> , 2012
		FE, FL, FS, FF and FU	RIL's	180	25,313 SSRs	62	Tang <i>et al.</i> , 2015
		, FL, FS, FE, FU and FC	F <sub>2</sub>	94	123 AFLPs	43	Cuming <i>et al.</i> , 2015
		2	Fiber and agronomical	SCY, LY, LP, BW, SI, FMT, PER, WF,WT, FF, FL, FE and FS	RIL's	188	141 SSRs
BW, LP, FF,ES, FU, DFF and DFN	F <sub>2</sub>			60	50 EST, 18 EST-SSR, 36 SSRs and 64 AFLP	81	Samer <i>et al.</i> , 2015
3	Yield and fiber	SCY, LI, SI, LY, no. of seeds per boll, FS, FL and FF	F <sub>2</sub>	69	834 SSRs, 437 SRAPs, 107 RAPDs, 16 REMAPs	57	He <i>et al.</i> , 2008
		FS, FL, FF, FE, LP, SI, NB, SCY and LY	RIL's	258	2131 SSRs	53	Shen <i>et al.</i> , 2007
		LI, SI, LY, SCY, NSB and FS	F <sub>2</sub>	69	834 SSRs, 437 SRAPs, 107 RAPDs and 16 REMAPs	52	He <i>et al.</i> , 2007
		NB, BW, SI, LP, LI, SCY, LY, FL, FS, FF, FE and FU	4WC and inbred lines	280	6123 SSRs and EST-SSRs	31	Qin <i>et al.</i> , 2008
		SCY, LY, NB, BW, LP, SI, LI and FBN	RIL's and IF <sub>2</sub>	180	2675 EST-SSRs	111	Liu <i>et al.</i> , 2012
		PH, FBN, BW, LP, LI, SI, LY, FL, FS, FE, FF and FU	<i>G. hirsutum</i> accessions	81	121 SSRs	180	Zhang <i>et al.</i> , 2013
		SCY, LY,LI, BW, FL, FS, FU	BILs	146	2,041 SSRs	67	Yu <i>et al.</i> , 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
Fiberstrength	qFs1	Zhang et al 2003
Fiberlength	qFL-D2-1	Wang <i>et al.</i> , 2006
CMS	Rf <sub>1</sub> , Rf <sub>2</sub>	Lan et al.1999 Liu et al.2003, Zhang et al.2005
GMS	ms <sub>5</sub> , ms <sub>6</sub> , ms <sub>15</sub>	Chen <i>et al.</i> ,2009
Fiber development	Li <sub>1</sub> ,Li <sub>2</sub> ,N <sub>1</sub> ,n <sub>2</sub> ,Fz,ha N <sub>1</sub> , n <sub>1</sub> , n <sub>2</sub>	Rong <i>et al.</i> ,2007
Leaf shape	L <sub>2</sub> ,P <sub>1</sub> ,Y <sub>1</sub>	Song <i>et al.</i> ,2005;Guo <i>et al.</i> ,2006;
	t <sub>1</sub> ,T <sub>1</sub>	Wright <i>et al.</i> ,1999
Glandless	gl1, gl2, gl3, gl4, gl5, and gl6	Pauly, 1979
Gladness	Gll and Gl2	Lee, 1965; McCarty <i>et al.</i> ,1996
Root-knot nematode resistance	rkn <sub>1</sub>	Wang <i>et al.</i> ,2006, Shen <i>et al.</i> ,2006
Blight resistance	B <sub>2</sub> ,B <sub>3</sub> ,B <sub>12</sub>	Wright <i>et al.</i> ,1998
Fusarium resistance	FWR	Wang <i>et al.</i> ,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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