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Genetic Improvement of Cotton through Molecular Breeding

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Abstract

Cotton is one of the most important cash crops in India and is the second largest producer of raw cotton in the world. Even after several economical transformations and technical advances in the synthetic fibers, cotton is still the most preferred fiber for its comfort and simplicity. Fiber from cotton (*Gossypium hirsutum* and *G. barbadense*) is a major product in the world economy. It is a botanically unique plant as it is a perennial allotetraploid derived from diploid Gossypium species, one of which does not produce lint, which is grown as an annual row crop. Cotton has been an economic mainstay in both developed and developing countries and there is a huge demand for improved raw cotton in global textile industries due to their modernization. Genetic improvement of cotton production through conventional breeding has shown slow progress due its complex genetic inheritance. Recent advances in transcriptome profiling, functional genomics, proteomics and metabolomics approaches, coupled with molecular marker-assisted breeding, MAS and transgenic technology have made significant contributions in enhancing the efficiency of cotton breeding; these methods are collectively referred as molecular breeding. MAS is used to transfer specific elite allele at a target locus from a donor line to a recipient line. The Efficiency of MAS depends on the heritability of the target trait. DNA markers allow us to identify the allelic composition of genotypes in a segregating population. The main advantages of using DNA markers vs. conventional selection are to accelerate the fixation of recipient alleles at non-target regions and to identify the genotypes containing crossovers close to target genes. Efforts to link fiber quantitative trait loci, QTLs, and expression of genes involved in fiber development with molecular breeding tools provide novel targets for the development of desirable cotton fiber and economically and agronomically important traits. Further it could be concluded that the range of markers along with QTLs provide innovative tools in the cotton genomics era.

Keywords: Cotton, MAS, Genomic mapping, Markers, Genetic Improvement.

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important fiber crop of the world and commonly known as white gold. Cotton cultivation in India is a source of livelihood for a considerable share of the farming community. Besides, the king of textiles, provide employment through textile mills and spinning mills to people in many parts of the country. A positive trend could be observed in the area, production and yield of cotton over the years. In particular, the productivity led growth in production witnesses the development of research activities which provides high yielding varieties and suitable efficient resource utilization techniques (Niranjan et al, 2017). History of cotton ink back to civilization of the Mohen Jo Daro as depicted by excavations (Khan, 2003). The area under cotton cultivation in India has almost doubled from about 4.4 million ha. at the time of independence (1947-48) to about 9.0 million ha. in 1990s. Simultaneously, the production has also increased more than six fold from 25 lakh bales to 160 lakh bales (CICR, 2000[a])

Aim of Study

To discuss the advanced molecular techniques in cotton breeding & Improvment

Review of Literature

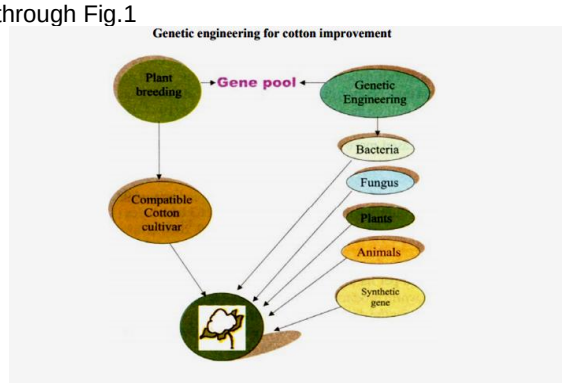
Cotton refers to those species of genus *Gossypium* that bears the spinnable seed coat fibres. There are about 42 species of this genus, out

of which only four species viz., *Gossypium arboreum, G. herbaceum, G.hirsutum* and *G.barbadense* are cultivated and rest are recognized as wild. The first two species i.e. *G. arboreum* and *G. herbaceum* are cytologically diploid (2n=2x=26) and native of Old World, termed as either Desi or Asiatic cotton. The remaining two i.e. *G .hirsutum* (American/Upland cotton) and *G. barbadense* (Sea land/ Egyptian or Tanguish cotton) are cytogentically tetraploids (2n=4x=52) and referred as New World Cotton (Ujjainkar,2006). In India, all four cultivated species viz., *G. hirsutum, G. barbadense, G. herbaceum, and G. arboretum* are grown whereas; *G. hirsutum* occupies the largest area among the four cultivated species grown and contributing alone 90 per cent to the global cotton production (Singh and Singh, 1999) and (Rakshe et al, 2019).

Cotton is the most important source of natural fibres and plays a dominant role in country's agrarian and industrial economy. Now-a-days, genetic enhancement or pre-breeding is gaining increasing ground as an importance in all the major crops. With the renewed interest and emphasis in Plant Genetic Resources (PGR) activities, it has become increasingly important to utilize the collected genetic diversity. Genetic enhancement plays an important role in utilizing unadapted and unutilized germplasm collections and creating vast genetic variability for development of productive cultivars / hybrids.

Plant breeders select those plants, which looks phenotypically more promising due to the presence of desirable traits. Most of the traits are controlled by polygenes with complex nonallelic quantitative effects and environmental interactions. In most cases, despite the fact that biometrical genetics reveals the presence of additive or non-additive effects on loci involved in the inheritance of quantitative trait, a specific locus may not be detected (Edwards et al, 1987). Tightly linked loci with desired trait can support plant breeding program by rapid introgression of quantitative trait loci (QTL) using associated molecular markers (Van Esbroeck, 1998). Genomic region having genes of interest for a particular trait is designated as QTL (Quantitative Trait Loc). QTL analysis involves partioning of genetic variation in single component. So, DNA-based molecular markers provide a tool to plant breeders for the selection of desirable plants based on genotype instead of phenotype.

The expression of gene(s) individually their interaction with the climatic factors and agronomic measures can determine the cultivar adaptability (Collins et al, 2008). Selection of new plant varieties with the desirable traits under given environmental conditions and cultural practices is the fundamental basis of plant breeding (Collard and Mackill, 2008), genetic variability produced in germplasm as a result of selection, which alter the inheritance pattern of the traits, is quite useful to screen and select the cultivars for required traits. New cultivars have been developed by exploiting genotypes with enormous variation (Budak et al, 2004). Rapid changes are needed in agricultural production, and biologically diverse as well as low-input novel farming systems must be developed and employed. There is also a need for new crop varieties that are having characterstics viz., fitting-in to global climate change in the present era, adapted to bio-diverse farming systems, and finally giving more products to farmers and eventually to consumers. The genetic engineering techniques employed for cotton improvement can be illustrated through Fig.1



(CICR, 2000[a])

Genetic engineering and newer transformation methods allow any gene form any source to be incorporated into the cotton genome, whereas cross breeding is restricted to compatible cotton cultivars Genetic engineering provides an alternate and powerful method for gene transfer from any organisms into cotton, whereas cross breeding is restricted to compatible cotton cultivars. The transferred foreign gene(s) integrates efficiently in to the plant chromosome. Then the integrated traits are inherited and expressed like any other plant genes. Thus, there is potential to improve cotton insect pest resistance traits (which is unique and extremely desirable at the present time), herbicide tolerance, fibre characteristics, oil content, tolerance to environmental extremes and even more fundamental physiological processes such as water and nutrient balance. The wealth of interesting genetic, physiological and agronomic problems available for study in cotton ensures that, there will be a rapid transition from the molecular analysis of gene structure to transform methods in more biological and agricultural applications (CICR, 2000[a]).

Molecular markers are the firm landmarks in the genome of an organism rather than the normal genes because mostly they do not have the biological impacts and may or may not relate with phenotypic expression of a trait. The development of the DNA markers is simple due to the availability of large scale genomic database In plant breeding, these markers are very helpful in recognition, characterization, identification of genetic variations, marker assisted selection (MAS), linkage mapping, and genomic fingerprinting, to remove linkage drag in backcrossing and to identify the traits which are not easy to measure by visual observation. Molecular marker technologies can be classified into hybridization based, PCR based, and sequenced based markers on the basis of their working mechanism. Among these, PCR-based markers, that is, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats

microsatellites (SSRs), and inter simple sequence repeats (ISSRs), represent the major class of markers in cotton genomics due to their high utility and exploitation. The comparison of different aspects of generally used molecular markers in cotton crop is given in Table 1(Malik et al., 2014)

Table 1: Comparison of marker systems in cotton

Marker	Template DNA quantity	Template DNA quality	Genetics	Cost	Reliability
RFLPs	High	High	Codominant	High	High
RAPDs	Low	High	Dominant	Low	Low
ISSRs	Low	Medium	Dominant	Low	Medium
SSRs	Low	Moderate	Codominant	Low	High
AFLPs	Medium	Moderate	Dominant	Moderate	High
SNPs	Low	High	Codominant	Low	High
GBS	Low	High	—	Low to moderate	High

Tetraploid genome of cotton is relatively large and contains about 2200–3000 Mb of DNA (Arumuganathan and Earle, 1991 and Paterson and Smith, 1999). The intra-specific DNA polymorphism is low in this species (Tatineni et al, 1996 and Brubaker and Wendel, 2001), which makes it a challenging crop for development of molecular markers. There is an undeniable need for highly polymorphic molecular markers if progress in plant breeding is to be made using marker-assisted breeding programs. Many extraordinary reviews have been written about the different classes of molecular markers used in plants and their application in construction of linkage map, QTL analysis and marker-assisted selection (Young, 1994 and Agarwal et al., 2008). The analysis of the evolution of molecular marker technologies in cotton genetics is an important objective of this review paper along with discussion over the points viz., genetic diversity in the wild and cultivated cotton gene pools, and QTL mapping and marker assisted selection activities in cotton. The QTLs identified in cotton germplasm using different marker technologies are summarized in Table 2.

Table: 2 The QTLs identified in cotton germplasm using different marker technologies

Traits	Descriptor	Population	Size	Marker (number and type)	QTLs number	Reference
Fiber quality	FS, FL and FF	F_2	171	RFLPs and 85 RAPDs	13	(Kohel et al., 2001)
	FS	F_2	186	217 SSRs, 800 RAPDs UBC and 1040 OPERON	2	(Zhang,et al 2003)
	LY, LP, SW, NS, UQ, SF, FL, FE, FT, FF and IF	F_2	120	144 AFLPs, RFLPs and 150 SSRs	28	(Mei et al, 2004)
	FS, FE, FL, FU, LP and FF	F_2	117	290 SSRs and 9 AFLPs	16	(Zhang et al, 2005)
	FF	BC_3F_2	3,662	262 RFLPs	41	(Draye et al, 2005)
	FL, FLU and SFC	BC_3F_2	3,662	262 RFLPs	45	(Chee et al, 2005)
	FS, FE, FU, FL and FF	RIL's	270	7508 SSRs, 384 SRAPs and 740 IT-ISJs	13	(Zhang et al, 2009)
	FL, FS, FF and FE	F_2	—	1378 SSRs	39	(Shen, 2005)
	FS, FL, FF, FMT, FE and SFI	RIL's	180	4106 SSRs, AFLPs, RAPDs and SRAPs	48	(Wang et al, 2006
	FE, FL, FS, FF and FU	CP	172	16052 SSRs	63	(Zhang et al, 2012)
Fiber and agronomical	SCY, LY, LP, BW, SI, FMT, PER, WF, WT, FF, FL, FE and FS	RIL's	188	141 SSRs	56	(Wu et al, 2009)
	FS, FL, FF, FE, LP, SI, NB, SCY and LY	RIL's	258	2131 SSRs	53	(Shen et al, 2007)
Yield and fiber	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 et al., 2008)

	SCY, LY, NB, BW, LP, SI, LI and FBN	RIL's and IF2	180	2675 EST-SSRs	111	(Liu et al, 2012)
	PH, FBN, BW, LP, LI, SI, LY, FL, FS, FE, FF and FU	*G. hirsutum* accessions	81	121 SSRs	180	(Zhang., et al, 2013)
	LI, SI, LY, SCY, NSB and FS	F_2	69	834 SSRs, 437 SRAPs, 107 RAPDs and 16 REMAPs	52	(He et al, 2007)
Morphological	LBNO, SL1, L1, W1, L2, W2, L3 and W3	F_2	180	261 RFLPs	62	(Jiang et al., 2000)
	EM	F_2 and F_3	—	4083 SSRs	54	(Li et al., 2013)
	NFB	F_2	251	1165 SSRs	5	(Guo et al, 2008)
Plant architectural	PH, FBL, FBN, FBA, FBL/PH and NMUB	RIL's	180	2130 SSRs, 2 RAPDs and 1 SRAP	16	(Wang et al., 2006)

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 plant, 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, EM: early maturity, NMUB: lower, middle, and upside boll number, NFB: node to first fruiting branch, LBNO: lobe numbers, SL1: sublobe number on the main lobe, L1: main-lobe length, W1: main-lobe width, L2: second-lobe length, W2: second-lobe width, L3: third-lobe length, W3: third-lobe length, PER: perimeter, WF: weight fitness, WT: wall thickness, FBL/PH: ratio of fruit branch length to plant height, RIL's: recombinant inbred lines, IF2: immortalized F2s, 4WC: four way cross, CP: composite cross, and BC_3F_2 = backcross families.

GENETIC ENGINEERING FOR FIBRE QUALITY

Textile industry needs are changing considerably as a result of innovations of synthetic fibre industry. Because of their ability to provide a wide range of improved fibres in a timely fashion has enabled them to capture 62% of the textile market, while cotton's share has shrunk to 32%. To address this challenge, strategies have been devised to improve existing fibre properties such as length, strength and micronaire value and more importantly to add new properties to fibre (e.g., dye - binding properties). Works are being carried out to determine the biochemistry and biology of fibre quality and correlate the relationship of fibre quality to yield and maturity. The fibre biochemistry and biology are used to identify "genes" that regulate fibre quality and thereby develop new approaches to select for cotton varieties with desirable yield and fibre characteristics.

The improvement of fibre quality can be possible through following approaches :

(a) Select a protein that is likely to have an effect on fibre development determines if genetic variation for this protein exists and associated that variability with a specific fibre trait.

(b) Use of multivariate analysis and discriminate functions to test plant biochemical and morphological traits for their association with fibre traits and build a model which correlates plant trait with traits for fibre quality.

(c) Identify Mendelian markers (e.g. RFLP, RAPD, AFLP, Isozyme etc.), such that regions of chromosomes can be followed during inheritance and associate with quantitative trait loci.

The properties of cotton fibre arise from the manifestation of thousands of genes in cotton. The conventional cotton breeding, part of the gene pool from one cultivar is exchanged with that of another compatible cotton cultivar. However, their diversity of traits among different cotton cultivars is limited. Thus there is only a narrow range of properties that can be enhanced through plant breeding and new properties from other organisms cannot be added. Recombinant DNA technology and new transformation methodologies can overcome this limitation. The critical task is to identity genes that can modify relevant fiber properties (CICR, 2000[a]).

Modification of fibre properties through genetic engineering, in addition to transformation capabilities, appropriate genes and promoters are required. Promoters are DNA elements that direct the expression of genes in tissues. Tissue-specific promoters activate genes in a tissue - specific manner. A number of fibre-specific promoters from cotton have demonstrated that it can be used to drive expression of genes in a fibre-specific manner in transgenic cotton. Of the many hundreds of potential genes available from various sources, only a few have actual value in fibre modification. Genes that would be useful include hormone genes from *Agrobacterium*, biopolymer genes from *Alcaligenes eutrophus* and genes encoding several cell wall protein (CICR, 2000[a]).

Cotton Genome Mapping

The genus Gossypium comprises of a total of 49 species which includes four domesticated species comprising of the new world allopolyploids G. hirsutum and G. barbadense (2n = 52) and the old world diploids G. arboreum and G. hirsutum (2n = 26).

Cotton genome has a total recombinational length of about 5200 cM (centi Morgan, cM = 400 kb) across the 2200-3000 Mb of DNA. The cotton genetic map constructed through crosses of G. hirsutum x G. barbadense comprised of 705 loci and 41 linkage groups spanning 4675 cM (CICR, 2000[b]).

The levels and patterns of RFLP variation across gene pools in cotton were examined at Texas through the use of 1376 DNA probes and four restriction enzymes to understand the variation among and within tetraploid species. A total of 462 loci were scored as co-dominant alleles. The levels of DNA polymorphism among the tetraploid species were found to be high. It was seen that the average G .Barbadense accession was comprised of 8.9% G .hirsutum alleles and that Pima cultivars (7.3%) had fewer G .hirsutum alleles than sea island (9.0%) or Egyptian cotton (9.6%). Mapping is ideally done through interspecific crosses where polymorphism is high. Biochemical and molecular markers have also been used in mapping the cotton genome. However, only 24 out of the 59 biochemical markers identified were found to be polymorphic. Only a few traits such as phosphoglucomutase 7 (pgm7), heat shock proteins and a-amylase were mapped to linkage groups and localised on chromosomes 10 and 12 (Saha and Stelly, 1994). Fluorescence *in-situ* hybridisation (FISH) is a molecular cytogenetic tool which was used to reveal a homeologous nuclear organising region (NOR) on chromosomes 26 and 16 (Stelly et al, 1992). So far only thirteen linkage groups could be associated with specific chromosomes (CICR, 2000[b]).

To make the best use of markers in cotton breeding programmes, it is necessary to isolate a large number of markers to select informative markers for all regions of the chromosome or to detect more rapidly evolving regions of the chromosome. In addition the identification of SSR (simple-sequence repeats) based markers can be used to identify cotton cultivars through generation of fingerprints as well as for marker assisted breeding.

A significant number of simple sequence repeat loci have been mapped on the cotton genome comprising of the most abundant poly (A) followed by (AT)n, (GA)n and (CA)n. Genetic mapping of 13 SSRs identified 20 polymorphic loci on 12 different linkage groups and all the 16 SSRs that were tested amplified DNA fragments in both A and D genome diploid progenitors of the cultivated AD genome (Paterson and Smith, 1999). With the existing markers it is possible to detect the introgression events such as transfer of genomic regions from G. hirsutum to G. barbadense and traits such as verticillium wilt and bacterial blight resistance; nectariless leaves; restoration of cytoplasmic male sterility and improved fibre quality.

Conclusion

Markers assisted selection is one of the widely used breeding strategies to improve traits including cotton fiber quality traits with a multifaceted genetic basis. Several genes associated with fiber development have been reported to function at different stages of fiber development in many articles. In past decades, thousands of QTLs in diploid and tetraploid cotton species have been identified in populations developed by interspecific crosses between parents differing in fiber characteristics. The genomic sequences of cotton provide precious resources to develop high-density SSR or SNP based genetic maps. Establishing linkage between phenotype and genotypic interactions, the identification of stable QTLs lays a basis for fine mapping to dig out the related genes.

Developing reliable markers, which will work in different populations and utilized in the breeding to enhance selection efficiency, is a very important step for breeding. Markers should allow desired genotype selection because of their tight linkage to the trait of interest. On the other hand, emerging technologies like high- throughput marker systems and marker- based selection methodologies have been developed, and are currently being used efficiently in cotton breeding. It is also promising that some economically important traits like fiber quality, yield, Verticillium wilt resistance, cotton leaf curl virus, drought tolerance, nematode resistance can be enhanced by using MAS. Genetic diversity can also be evaluated by using DNA markers before starting breeding program. Tremendous efforts have been carried for studying genetic diversity from genotypic and phenotypic aspects in germplasm accessions of cotton. Many QTLs related to economical traits have been discovered. It is an emerging concern that efforts should be made for the utilization of molecular breeding methodologies to enhance cotton productivity, which can be enhanced through the recent developments in NGS.

Moreover, highly saturated maps are useful for determining genetic manipulations from heredity perspectives, and SNPs are the best for this purpose. These markers along with QTLs provide innovative tools in the cotton genomics era. With genomic and bioinformatics approaches, it is more feasible to retrieve target QTL regions. The integration of transcriptomic analysis and QTL mapping can reveal more concrete information about fiber development mechanism. These studies should be improved in corresponding cotton genomes and among the populations developed by interspecific crosses. Cotton genomes, DNA markers and transcriptomic studies can play a major role in dissecting the mechanisms underlying fiber development to cultivate superior varieties with improved cotton fiber quality.

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