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ENHANCEMENT OF YIELD AND QUALITY OF COTTON (*GOSSYPIUM HIRSUTUM L.*) THROUGH DIFFERENT MOLECULAR MARKERS

ABBAS A[1], REHMAN AU[1], ARSHAD A[2], RAZA GM[3], UMAR M[4], BUKHARI MS*[5]

[1]*Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab, P.O BOX. 54590, Lahore, Pakistan*
[2]*Department of Seed Science and Technology, University of Agriculture Faisalabad P.O BOX, 38000, Faisalabad, Pakistan*
[3]*Department of Agronomy, University of Agriculture Faisalabad P.O BOX, 38000, Faisalabad, Pakistan*
[4]*Department of Soil Science, University of Agriculture Faisalabad P.O BOX, 38000, Faisalabad, Pakistan*
[5]*Agricultural Research Station Bahawalpur, P.O BOX, 63100, Bahawalpur, Pakistan*
**Correspondence author email address: shahjhanbukhari@gmail.com*

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Abstract *Cotton (Gossypium hirsutum L.) is a member of the Malvaceae family and the Gossypium genus, which contains 50 different species. Only four of these species, however, are commercially farmed. This study aims to evaluate the genetic diversity of wild and cultivated cotton gene pools, as well as QTL mapping and marker-assisted selection activities in cotton genetics. Various marker-based approaches, including RAPD, ISSR, AFLP, SSR, and SNP analysis, have been used to investigate genetic diversity, genotype correlations, and map saturation in cotton. These technologies have also assisted genome-wide association studies (GWAS) and the finding of quantitative trait loci (QTLs). Furthermore, novel approaches such as linkage disequilibrium, association mapping, and genomic selection are applied to classic ideas such as genetic variation, QTL mapping, and marker-assisted selection (MAS). These genomic technologies can boost cotton productivity and meet global demand for high-yielding, high-quality cotton fiber by incorporating additional omics resources.*

Keywords*:* *NGS; MAS; QTL mapping; genome; omics*

Introduction

Cotton is a Malvaceae and *Gossypium* plant. Cotton has 50 species 45 diploid and 5 allotetraploid. These species inhabit Africa, Central and South America, the Galapagos Islands, the Indian subcontinent, Australia, Arabia, and Hawaii (Hu et al., 2021; Huang et al., 2021). The A, B, E, and F genomes are mostly found in Asia and Africa. The D genome is mostly found in the United States, and the C, G, and K genomes are mostly found in Australia. Growing four of the 50 species is possible: *G. hirsutum, G. barbadense, G. arboreum, and G. herbaceum* (Karaca et al., 2020). These farmed species have 52 or 26 chromosomes (2n) and the genotypes AADD, AADD, A2A2, and A1A1 (Sabev et al., 2020).

In the past, about one to two million years ago, tetraploid cotton became domesticated by crossing a D genome donor species (*Gossypium Raimondi and Gossypium gossypioides*) with an A genome donor species (*Gossypium herbaceum and Gossypium arboreum*) and then going through polyploidization (Abbas and Muqaddasi, 2021). This resulted in the production of a progenitor allotetraploid species known as "AD," which subsequently resulted in the emergence of the "AD" tetraploid species known as *Gossypium hirsutum L.* (also known as Mexican cotton) and *Gossypium barbadense L.* (also known as Sea Island cotton or Egyptian cotton) (Hu et al., 2021). Although *G. hirsutum* is responsible for 90 percent of the world's cotton production, *G. barbadense* is responsible for 8 percent (Hu et al., 2019). The remaining two percent is supplied by *G. herbaceum* (also known as Levant cotton) and *G. arboreum* (also known as Tree cotton) (Aaliya et al., 2016; Suomela et al., 2023; Viot, 2019).

Cotton has four sets of chromosomes that make up its genome, which is between 2200 and 3000 megabytes. Because DNA from individuals of the same species doesn't very much, it's hard to make genetic markers that work well for breeding cotton (Ahmad et al., 2012; Ahmad et al., 2021; Ali et al., 2017; Aslam et al., 2020). While this is still the case, creating highly polymorphic genetic markers is necessary for progress in plant breeding using marker-assisted programs (Ahmar et al., 2021; Ali et al., 2015; Ali et al., 2013; Aslam et al., 2020). Much research has been done on genetic markers, which are used to make linkage maps, study quantitative trait loci (QTL), and use marker-assisted selection in plants. The goals of this study are to (i) look at how molecular marker

technologies have changed over time in cotton genetics**,** (ii) look at the genetic variety that exists in both wild and cultivated cotton gene pools**,** and (iii) give an overview of QTL mapping and marker-assisted selection in cotton (Kushanov et al., 2021).

Table 1 Evaluating of Different Markers in Cotton.

Types of Markers	DNA Quantity	DNA Sample	Study of Genetics	Price of markers	Accuracy of markers	Citations
RFLP	Higher	Higher	Codominant	Higher	Higher	(Amiteye, 2021; Salisu et al., 2018)
RAPD	Lower	Higher	Dominant	Lower	Lower	(Mahmood et al., 2021)
ISSR	Lower	Middle	Dominant	Lower	Middle	(Zaki and Hussein, 2023), (Baran et al., 2023)
SSR	Lower	Middle	Codominant	Lower	Higher	(Zhang et al., 2020), (Kuang et al., 2022)
AFLP	Middle	Middle	Dominant	Moderate	Higher	(Niu et al., 2022), (Baran et al., 2023)
SNP	Lower	Higher	Codominant	Lower	Higher	(Park et al., 2021), (Sabev et al., 2020)
GBS	Lower	Higher	—	Lower to moderate	Higher	(Diouf et al., 2018), (Diouf et al., 2017)

Cotton Molecular Marker Technology innovations

Researchers think that molecular markers are more steady than genes because they don't have a big effect on biological processes and might not affect phenotypic features (Ormel et al., 2019). Expanding genetic databases has made it easier to make these markers, which are very important for mapping genomes (Ali et al., 2016; Ali et al., 2010a; Yu et al., 2021). Their uses in plant breeding include finding and studying genetic differences, as well as marker-assisted selection (MAS), linkage mapping, genomic fingerprinting (Shimizu et al., 2020), getting rid of linked genes during backcrossing, and finding traits that are hard to see. For example, molecular markers can be hybridized, polymerase chain reaction (PCR), or sequence-based markers, depending on how they work. PCR-based markers like RAPD, AFLP, SSRs, and ISSRs are widely used in cotton genomics because they work well and can be used in several different approaches (Sabev et al., 2020).

Utilizing Hybridization for DNA Markers

Restriction fragment length polymorphism (RFLP) markers discover differences in the sizes of DNA fragments produced by restriction enzymes (Tarach, 2021). These markers detect variations in the length of DNA fragments generated by even a single base mutation in the recognition sequence of a restriction enzyme. They use cDNA or manufactured oligonucleotides as probes to construct DNA profiles and hybridize restricted DNA segments with a radioisotope-labeled probe (Cheng et al., 2022). RFLPs have been successfully employed to study crop-weed introgression and gene flow. They have also been widely used in cotton studies for population genetics, evolution, and phylogeny, with roughly 64% of cotton RFLP markers showing codominance. These markers have been quite useful in determining genetic diversity in upland cotton. The first cotton genome molecular map was created using 705 RFLP loci grouped into 41 linkage groups (Ujjainkar). In marker-assisted selection (MAS), an RFLP marker was also employed to validate a bacterial blight resistance allele (Chukwu et al., 2019). RFLP analysis, however, has been mainly supplanted by more efficient polymerase chain reaction (PCR)-based markers due to its complexity, time-consuming nature, and high cost.

Genetic Marker Identification Using PCR

Polymerase chain reaction (PCR) replicates small amounts of DNA without a living organism. The procedure uses a DNA polymerase like Taq to read and synthesis a new strand of DNA in the 5-3 direction utilizing dNTPs. PCR can amplify small amounts of DNA and damaged DNA (Burke and Lupták, 2018). PCR is a widely used process that consists of three major steps: denaturation, annealing, and extension. The detection of the resultant PCR products is frequently accomplished using agarose or polyacrylamide gels. This method is extensively used in genetic diversity study and DNA marker identification. Because of PCR's simplicity and high success rate, many approaches for producing PCR-based molecular markers have been developed.

Utilizing Random Amplified Polymorphic DNA (RAPD) for Genetic Analysis

RAPD is a PCR method used to detect genetic changes caused by deletions or recombination events between specific areas of DNA. This method entails the amplification of DNA fragments with random 10-base pair primers, a GC content of at least 40%, and the lack of palindromic sequences (Kadri, 2019). A specific product can be amplified if the primers successfully bind to the target DNA regions. RAPD is used to study genetic variability in population (Niu et al., 2019), DNA profiling (Mnookin, 2017) and genotype relatedness (Handi et al., 2017). In cotton, RAPD has been used to distinguish genotypes resistant to jassids, aphids, and mites (Arora et al., 2017) and to identify the male sterility gene marker (R-6592) (Sabev et al., 2020). In cotton, RAPD is used to evaluate genotype correlations (Ghuge et al., 2018), discover stomatal conductance QTLs

(MAGWANGA et al., 2020), and create linkage maps.

ISSR Analysis

ISSRs amplify DNA segments between identical SSRs in opposite orientations (Simair and Simair, 2020). As Sabev et al. stated in 2020, one method for detecting polymorphism in inter-SSR loci is using primers (16-25 bp) uniquely complementary to a single SSR sequence. These primers can have sequences ranging from di- to penta-nucleotide lengths (Wang et al., 2017). ISSR primers have a brief 1-4 base flanking stretch, specifically binding to the 3' or 5' end. Primers anchored at the 3' end produce more defined bands than those anchored at the 5' end (He et al., 2023). The ISSR method combines AFLP and SSR benefits with RAPD universality. ISSR primers' longer sequence allows for a higher annealing temperature, improving band repeatability over RAPDs (Iqbal et al., 2023). ISSRs also create more pieces per primer than RAPDs. ISSRs are better than RAPDs for assessing crop species genetic diversity. ISSR uses vary by genome SSR diversity and frequency (WAHAB, 2017a). Gene tagging, genetic diversity analysis, and SSR motif discovery are common uses of this technology in plant improvement research.

Exploring Amplified Fragment Length Polymorphism (AFLP) - A Useful Tool in Genetic Studies

As a solution to RAPD repeatability, AFLP markers were developed (WAHAB, 2017b). This approach uses PCR to detect many loci in one reaction (Li et al., 2017), demonstrating high genome polymorphism (Ulloa et al., 2017). As well as the GC content and genome size, the number and theme of chosen nucleotides in the primer can change the AFLP amplification counts (Ali et al., 2014; Ali and Malik, 2021; Ali et al., 2010b; Iqbal et al., 2023). This method works well for studying genetic variety, making fingerprints, and labeling different aspects of crop, seed, and fiber quality (Iqbal et al., 2021). Because AFLP markers are very common and spread out widely, they can be used to map genes. AFLP has been used in cotton linkage maps, genetic variation analyses, and map saturation studies, among other things (Iqbal et al., 2021; Kumar et al., 2022).

SSR Marker

Microsatellites' short, repeated nucleotide sequences are prevalent in both coding and noncoding genomes. Different types of transferable markers are used to study genetic variation, make molecular maps, and choose markers to help with selection. Over 1000 SSR primers produced for cotton research have made microsatellites useful in cotton genetics research. Cotton Gen contains these primers and mapping information. Functional genes are linked to EST-SSRs derived from expressed sequence tags. EST-SSRs have less polymorphism than traditional SSRs but are more transferable between species and can offer gene expression data. CAPS microsatellites use RFLP and PCR to detect mutations and polymorphisms. Physiological and biochemical gene product markers can examine complicated attributes and identify markers. Microsatellites like EST-SSRs and CAPS are useful for understanding genetic variability and gene expression in cotton genetics studies.

Exploring Sequence-Based DNA Markers in Genetics SNP analysis

These are differences in an individual's genetic material made up of four nucleotides: A, T, C, and G. SNPs can be found in coding, non-coding, and intergenic regions, among other places. These differences may be the same or different words, and they can cause changes in an organism's appearance. Gene studies like linkage mapping, map-based cloning, and marker-assisted selection can be used because they are common and codominant. Finding SNPs in cotton, though, is hard because it has a small genetic basis and allotetraploid DNA (Hu et al., 2019). With the improvement of high-throughput sequencing tools, it is now possible to find many SNPs, even in species like cotton that don't have a lot of molecular studies or genetic variation. Many research projects have been done to look into and map SNPs in the Gossypium genome (Ali et al., 2018). Scientists worldwide have also successfully made a 70K Illumina Infinium genotyping assay-based SNP chip. This brand-new genotyping assay will help breeders, geneticists, and other experts worldwide with genetic studies, breeding, putting together genome sequences, and other things. In addition, Affymetrix is currently validating a Gene Chip cotton genome array with 239777 probe sets that encode 21485 cotton transcripts. This will soon be ready for commercial use. With global collaboration, SNP chip development sequences came from Gene Bank, db EST, and Ref Seq. These technologies will help fine map and uncover genes for essential economic features in cotton and enable genomic selection studies, enhancing cotton breeding efficiency.

GBS: Sequencing Genotyping methods

GBS stands for genotyping by sequencing, which is a way to simultaneously find and genotype single nucleotide variations (SNPs) in a genome. Designed to make the genome less complicated, this method works well and quickly. A simple method for making a GBS library is using a single restriction enzyme to grab the genome sequence between restriction sites (Wallace and Mitchell, 2017). When using GBS, it is important to pick the right restriction enzyme to eliminate copied parts of the genome. A methylation-sensitive restriction enzyme called "ApeKI" was used in the first GBS method for maize and barley to find the hypomethylated genome parts for sequencing (NYONGESA, 2017). A changed GBS method was also created, which uses two enzymes and a Y-adapter to create "uniform" GBS libraries. Adapter 1 and Adapter 2 were put on opposite ends of each piece. GBS is a flexible method that can find thousands of

SNPs in a single experiment. It can be used for many breeding goals, including population studies, genomic selection, genetic mapping, and characterizing genotypes (Shamshad and Sharma, 2018).

Improving crop enhancement using marker-based methods

Exploring Genetic Diversity in Cotton

In the 1990s, 80 RAPD markers were used to examine the genetic diversity of 16 homozygous top genotypes that were gained through inter-specific hybridization. With this method, *G. hirsutum* and *G. arboretum* could be told apart. In a different study, 45 RAPD primers were used to look at 31 Gossypium species, subspecies, interspecific crosses, and short-lived cotton genotypes. The results showed that the cotton leaf curl virus could not infect two races. AFLP was also used to compare diploid and tetraploid cotton species by looking at differences in the ribosomal RNA gene. AFLP has also been used to examine the genetic variety of wild animals, upland cotton, and their offspring. It has also been used to find out how *G. barbadense, G. arboretum, G. Raimondi, and G. hirsutum* are related genetically. It has been found that SSR markers can successfully identify transcribed genes and show high levels of polymorphism. This makes them useful for studying genetic diversity in cotton. Many studies have successfully used SSR markers to examine the genetic variation between cotton cultivars and species, and the plants are still growing. Next-generation sequencing and RNA-seq SNPs have been used to characterize genetic differences in cotton species. In F2 populations of upland cotton cultivars, the KAS Per assay targets specific SNPs and determines their Mendelian segregation ratio.

Cotton Production QTL Mapping for Key Economic Characteristics

Quantitative trait loci, or QTLs, are genome portions with genes connected to a certain quantitative feature. Understanding QTL mapping, also called finding and mapping QTLs, is important for understanding the connection between a phenotype and a marker's genotype. The identification of QTLs in cotton germplasm has been accomplished by utilizing various molecular marker technologies, including RFLPs and RAPDs. By way of illustration, RFLPs were utilized in earlier research to identify fourteen QTLs for characteristics connected to fiber. RFLP mapping has also been utilized to find quantitative trait loci (QTLs) for various characteristics, including the density of stem and leaf trichome, the amount of gossypol present, and the amount of chlorophyll present. Different genetic markers, like SSRs and EST-SSRs, have been used to map cotton quantitative trait loci (QTLs). Researchers have found many QTLs linked to important traits like plant design, yield, and fiber quality using these methods. The Cotton Gene database has 988 QTLs for 25 traits and can give you access to this information. This large collection contains useful information that can help develop new marker-assisted breeding methods for cotton.

Genome-Wide Association Studies of Cotton Genetic Links

Associate mapping, which is sometimes referred to as linkage disequilibrium (LD) mapping, is a technique that is employed to analyze the variance in complex traits. To reach this goal, we look at how the recombination patterns have changed over time and as the population has evolved. Using this approach, non-structured populations are subjected to phenotyping and genotyping to determine whether there are any relationships between characteristics and markers. Association mapping provides a greater range of recombination and higher resolution mapping than traditional linkage mapping. This is in comparison to the traditional linkage mapping. Abdullaev et al. (2017) this method has been effectively used in the field of cotton research, and it takes advantage of the genetic diversity that may be discovered in the collection of cotton germplasm from around the world. LD-based linkage mapping is increasingly being used, meaning that biparental QTL mapping is no longer the main focus. This is similar to the occurrence of other plant genetic resources simultaneously. Because of this modification, it is now possible to use the exsitu conserved genetic diversity that may be found in worldwide germplasm banks for cotton. Furthermore, to achieve effective association mapping in the cotton genome, a relatively minimal number of markers are required, comparable to the results obtained from other crops (Ademe et al., 2017; Ali et al., 2011). The tetraploid genome of cotton is an interesting part of study. With a total recombination length of about 5,200 cm and an average of 400 kb per cm, 5–6 cm LD blocks are all needed for association mapping for different traits. To achieve a successful and accurate association mapping, this would necessitate using a maximum of around one thousand polymorphic markers. Because of recent developments in genome sequencing technology make it possible to gather large genotypic datasets, making it easier to employ association mapping rather than QTL mapping (Zhang et al., 2023).

Improvement of cotton breeding through MAS

By using the genetics of a marker, plant breeders can choose plants with the features they want. This is called marker-assisted selection (MAS). Choosing plants with the right mix of certain genes is the most important part of plant breeding. Markers closely linked to these genes help breeders find plants with their desired genes. MAS works or doesn't work depending on the marker method, so making a good choice is important. Researchers have been using RAPD methods in MAS to raise seeds of different species, such as *G. sturtianum*, with and without glands in recent years (Hu et al., 2021). Studies have shown that DNA markers linked to the main QTL for fiber strength (QTLFS1) can be used in MAS to

strengthen commercial crops' fibers in segregating populations. To make it easier to find the main fiber strength QTL in BC1F4 upland cotton, some RAPD markers have also been changed into specific SCAR markers, like the SCAR 1920 marker (Sabev et al., 2020). It was also possible to find three more markers connected to the CBD gene after finding SNPs on chromosome 10. These markers can be used successfully in MAS to make cotton breeding programs less likely to get blue disease (Conaty et al., 2022; Waghmare, 2022).

Analyzing the preliminary version of the Cotton Genome and its importance

Recent progress in DNA sequencing has made it much easier to find genes and molecular markers linked to different features. This has opened up new ways to improve crops. By sequencing DNA, we can learn more about how different species in the Gossypium group are. The tetraploid cotton species ($2n = 4_e = 52$), namely G. hirsutum and G. barbadense, are thought to have come from an allopolyploidization event about 1-2 million years ago. During this event, a D-genome species was the pollen parent, and an A-genome was the female parent (Hao et al., 2017). It is important to know what each constituent genome is made of to fully understand how sub-genomes have changed over time and how they relate to each other in developed polyploid genomes. Cotton geneticists think that sequencing the D-genome father, G. Raimondi, is important for this main reason. G. Raimondi is the smallest species in the Gossypium genus. Its genome is only 880Mb (Wu et al., 2017), making up about 60% of the diploid A-genome and 40% of the tetraploid genomes (Khidirov et al., 2023). Putting together a physical map of the G. Raimondi genome has shown that it comprises parts that are high in both genes and repeats.

Future Aspect

Many nations rely on cotton cultivation for foreign exchange. Improving cotton fiber quantity and quality is a priority. Novel alleles from wild species and current molecular technologies are being introduced to improve economic features. The *G. Raimondii* and *G. arboreum* draft genomes are being sequenced to find significant features. These genomic resources can also identify high-throughput marker platforms such Select SNP arrays, which can distinguish desirable cotton genotypes and research genetic diversity and produce linkage maps. These markers are essential for variety development. QTL mapping has been used to discover cotton features such as fiber output and quality, drought tolerance, disease resistance, and insect resistance (Diouf et al., 2018). However, low marker density may make it difficult to clone causative genes. Molecular marker techniques are chosen based on dependability, statistical power, and polymorphisms. These techniques can spark a new "Green Revolution" in agriculture as they become more efficient and automated. More efficient DNA markers will be developed soon to help plant breeders and geneticists create cultivars that fulfill societal needs. Due to their availability and detection system improvements, SNP markers will impact MAS and mapping investigations (Sabev et al., 2020). Marker genotyping with GBS is predicted to grow in popularity. New markers and high-tech tools like DNA chips and microarrays will speed up the process of tracking and identifying genes in cotton, leading to faster and better varietal development.

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Declaration

Conflict of interest

There is no conflict of interest among the authors.

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