Journal of Physical, Biomedical and Biological Sciences
eISSN: 3005-6489
www.jpbab.com
J. Phys. Biomed. Biol. Sci., Volume, 3: 19

Review Article **Open Access**

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*

(Received, 17th June 2023, Revised 11th January 2024, Published 13th January 2024)

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.

References

Aaliya, K., Qamar, Z., Ahmad, N. I., Ali, Q., Munim, F. A., and Husnain, T. (2016). Transformation, evaluation of gtgene and multivariate genetic analysis for morpho-physiological and yield attributing traits in Zea mays. *Genetika* **48**, 423-433.

Abbas, A., and Muqaddasi, Q. H. (2021). Genomics-Assisted Breeding for Biotic Stress Syndrome Resistance in Cotton. *In* "Cotton Precision Breeding", pp. 113-136. Springer.

Abdullaev, A. A., Salakhutdinov, I. B., Egamberdiev, S. S., Khurshut, E. E., Rizaeva, S. M., Ulloa, M., and Abdurakhmonov, I. Y. (2017). Genetic diversity, linkage disequilibrium, and association mapping analyses of Gossypium barbadense L. germplasm. *PLoS One* **12**, e0188125.

Ademe, M. S., He, S., Pan, Z., Sun, J., Wang, Q., Qin, H., Liu, J., Liu, H., Yang, J., and Xu, D. (2017). Association mapping analysis of fiber yield and quality traits in Upland cotton (Gossypium hirsutum L.). *Molecular Genetics and Genomics* **292**, 1267-1280.

Ahmad, H. M., Ahsan, M., Ali, Q., and Javed, I. (2012). Genetic variability, heritability and correlation studies of various quantitative traits of mungbean (Vigna radiate L.) at different radiation levels. *International Research Journal of Microbiology* **3**, 352-362.

Ahmad, M., Ali, Q., Hafeez, M. M., and Malik, A. (2021). Improvement for biotic and abiotic stress tolerance in crop plants. *Biological and Clinical Sciences Research Journal* **2021**.

Ahmar, S., Ballesta, P., Ali, M., and Mora-Poblete, F. (2021). Achievements and challenges of genomics-assisted breeding in forest trees: From marker-assisted selection to genome editing. *International Journal of Molecular Sciences* **22**, 10583.

Ali, F., Ahsan, M., Ali, Q., and Kanwal, N. (2017). Phenotypic stability of Zea mays grain yield and its attributing traits under drought stress. *Frontiers in plant science* **8**, 1397.

Ali, F., Kanwal, N., Ahsan, M., Ali, Q., Bibi, I., and Niazi, N. K. (2015). Multivariate analysis of grain yield and its attributing traits in different maize hybrids grown under heat and drought stress. *Scientifica* **2015**.

Ali, I., Teng, Z., Bai, Y., Yang, Q., Hao, Y., Hou, J., Jia, Y., Tian, L., Liu, X., and Tan, Z. (2018). A high

density SLAF-SNP genetic map and QTL detection for fibre quality traits in Gossypium hirsutum. *BMC genomics* **19**, 1-18.

Ali, Q., Ahsan, M., Ali, F., Aslam, M., Khan, N. H., Munzoor, M., Mustafa, H. S. B., and Muhammad, S. (2013). Heritability, heterosis and heterobeltiosis studies for morphological traits of maize (Zea mays L.) seedlings. *Advancements in Life sciences* **1**.

Ali, Q., Ahsan, M., Kanwal, N., Ali, F., Ali, A., Ahmed, W., Ishfaq, M., and Saleem, M. (2016). Screening for drought tolerance: comparison of maize hybrids under water deficit condition. *Advancements in Life Sciences* **3**, 51-58.

Ali, Q., Ahsan, M., and Saleem, M. (2010a). Genetic variability and trait association in chickpea (Cicer arietinum L.). *Electronic Journal of Plant Breeding* **1**, 328-333.

Ali, Q., Ahsan, M., Tahir, M. H. N., Elahi, M., Farooq, J., Waseem, M., and Sadique, M. (2011). Genetic variability for grain yield and quality traits in chickpea. *International Journal of Agro-Veterinary and Medical Sciences* **5**, 201-208.

Ali, Q., Ali, A., Ahsan, M., Nasir, I. A., Abbas, H. G., and Ashraf, M. A. (2014). Line× Tester analysis for morpho-physiological traits of Zea mays L seedlings. *Advancements in Life sciences* **1**, 242-253.

Ali, Q., and Malik, A. (2021). Genetic response of growth phases for abiotic environmental stress tolerance in cereal crop plants. *Genetika* **53**, 419-456.

Ali, Q., Muhammad, A., and Farooq, J. (2010b). Genetic variability and trait association in chickpea (Cicer arietinum L.) genotypes at seedling stage. *Electronic Journal of Plant Breeding* **1**, 334-341.

Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon* **7**.

Arora, R., Kataria, S. K., and Singh, P. (2017). Breeding for insect resistance in cotton: advances and future perspectives. *Breeding Insect Resistant Crops for Sustainable Agriculture*, 265-288.

Aslam, S., Khan, S. H., Ahmed, A., and Dandekar, A. M. (2020). The tale of cotton plant: From wild type to domestication, leading to its improvement by genetic transformation. *American Journal of Molecular Biology* **10**, 91-127.

Baran, N., Shimira, F., Nadeem, M. A., Altaf, M. T., Andirman, M., Baloch, F. S., and Gültekin Temiz, M. (2023). Exploring the genetic diversity and population structure of upland cotton germplasm by iPBS-retrotransposons markers. *Molecular Biology Reports*, 1-13.

Burke, C. R., and Lupták, A. (2018). DNA synthesis from diphosphate substrates by DNA polymerases. *Proceedings of the National Academy of Sciences* **115**, 980-985.

Cheng, Y., Wang, N., Ren, Z., and Xu, C. (2022). Development of fluorescence-based nucleic acid blot hybridization method using Cy5. 5 labeled DNA probes. *Journal of Microbiological Methods* **197**, 106479.

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I., Oladosu, Y., Okporie, E., Onyishi, G., Utobo, E., Ekwu, L., and Swaray, S. (2019). Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (Oryza sativa L.). *Biotechnology & Biotechnological Equipment* **33**, 440-455.

Conaty, W. C., Broughton, K. J., Egan, L. M., Li, X., Li, Z., Liu, S., Llewellyn, D. J., MacMillan, C. P., Moncuquet, P., and Rolland, V. (2022). Cotton breeding in Australia: meeting the challenges of the 21st century. *Frontiers in Plant Science* **13**, 904131.

Diouf, L., Magwanga, R. O., Gong, W., He, S., Pan, Z., Jia, Y. H., Kirungu, J. N., and Du, X. (2018). QTL mapping of fiber quality and yield-related traits in an intra-specific upland cotton using genotype by sequencing (GBS). *International journal of molecular sciences* **19**, 441.

Diouf, L., Pan, Z., He, S.-P., Gong, W.-F., Jia, Y. H., Magwanga, R. O., Romy, K. R. E., Or Rashid, H., Kirungu, J. N., and Du, X. (2017). High-density linkage map construction and mapping of salt-tolerant QTLs at seedling stage in upland cotton using genotyping by sequencing (GBS). *International Journal of Molecular Sciences* **18**, 2622.

Ghuge, S., Mehetre, S., Chimote, V., Pawar, B., and Naik, R. (2018). Molecular characterization of cotton genotypes using SSR, ISSR and RAPD markers in relation to fiber quality traits. *Journal of Cotton Research and Development* **32**, 1-12.

Handi, S. S., Katageri, I. S., Adiger, S., Jadhav, M. P., Lekkala, S. P., and Reddy Lachagari, V. B. (2017). Association mapping for seed cotton yield, yield components and fibre quality traits in upland cotton (Gossypium hirsutum L.) genotypes. *Plant Breeding* **136**, 958-968.

Hao, M., Li, A., Shi, T., Luo, J., Zhang, L., Zhang, X., Ning, S., Yuan, Z., Zeng, D., and Kong, X. (2017). The abundance of homoeologue transcripts is disrupted by hybridization and is partially restored by genome doubling in synthetic hexaploid wheat. *BMC genomics* **18**, 1-12.

He, Q., Shang, X., Tian, R., Zhu, X., and Guo, W. (2023). An efficient and accurate droplet digital PCR method for rapid transgene copy number detection and homozygous identification in cotton (Gossypium hirsutum). *Industrial Crops and Products* **204**, 117284.

Hu, G., Grover, C. E., Jareczek, J., Yuan, D., Dong, Y., Miller, E., Conover, J. L., and Wendel, J. F. (2021). Evolution and diversity of the cotton

genome. *In* "Cotton precision breeding", pp. 25-78. Springer.

Hu, Y., Chen, J., Fang, L., Zhang, Z., Ma, W., Niu, Y., Ju, L., Deng, J., Zhao, T., and Lian, J. (2019). Gossypium barbadense and Gossypium hirsutum genomes provide insights into the origin and evolution of allotetraploid cotton. *Nature genetics* **51**, 739-748.

Huang, G., Huang, J.-Q., Chen, X.-Y., and Zhu, Y.-X. (2021). Recent advances and future perspectives in cotton research. *Annual review of plant biology* **72**, 437-462.

Iqbal, J., Altaf, M. T., Jan, M. F., Raza, W., Liaqat, W., Haq, I., Jamil, A., Ahmed, S., Ali, A., and Mehmood, A. (2023). Exploring genetic diversity in cotton genotypes using EST-SSR and ISSR markers: A comparative study. *Sarhad Journal of Agriculture* **39**, 800-814.

Iqbal, M. Z., Jamil, S., Shahzad, R., Bilal, K., Qaisar, R., Nisar, A., Kanwal, S., and Bhatti, M. K. (2021). DNA fingerprinting of crops and its applications in the field of plant breeding. *Journal of Agricultural Research (03681157)* **59**.

Kadri, K. (2019). Polymerase chain reaction (PCR): Principle and applications. *Synthetic Biology-New Interdisciplinary Science*, 1-17.

Karaca, M., Ince, A. G., and Reddy, U. K. (2020). Interspecific grafting between Gossypium hirsutum, G. barbadense and G. herbaceum lines. *Scientific Reports* **10**, 18649.

Khidirov, M. T., Ernazarova, D. K., Rafieva, F. U., Ernazarova, Z. A., Toshpulatov, A. K., Umarov, R. F., Kholova, M. D., Oripova, B. B., Kudratova, M. K., and Gapparov, B. M. (2023). Genomic and Cytogenetic Analysis of Synthetic Polyploids between Diploid and Tetraploid Cotton (Gossypium) Species. *Plants* **12**, 4184.

Kuang, Z., Xiao, C., Ilyas, M. K., Ibrar, D., Khan, S., Guo, L., Wang, W., Wang, B., Huang, H., and Li, Y. (2022). Use of SSR Markers for the Exploration of Genetic Diversity and DNA Finger-Printing in Early-Maturing Upland Cotton (Gossypium hirsutum L.) for Future Breeding Program. *Agronomy* **12**, 1513.

Kumar, P., Nimbal, S., Budhlakoti, N., Singh, V., and Sangwan, R. S. (2022). Genetic diversity and population structure analysis for morphological traits in upland cotton (Gossypium hirsutum L.). *Journal of Applied Genetics*, 1-15.

Kushanov, F. N., Turaev, O. S., Ernazarova, D. K., Gapparov, B. M., Oripova, B. B., Kudratova, M. K., Rafieva, F. U., Khalikov, K. K., Erjigitov, D. S., and Khidirov, M. T. (2021). Genetic diversity, QTL mapping, and marker-assisted selection technology in cotton (Gossypium spp.). *Frontiers in plant science* **12**, 779386.

Li, C., Unver, T., and Zhang, B. (2017). A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in Cotton (Gossypium hirsutum L.). *Scientific reports* **7**, 43902.

Magwanga, R. O., Lu, P., Kirungu, J. N., Cai, X., Zhou, Z., Agong, S. G., Wang, K., and Liu, F. (2020). Identification of QTLs and candidate genes for physiological traits associated with drought tolerance in cotton. *Journal of Cotton Research* **3**, 1-33.

Mahmood, T., Wang, X., Ahmar, S., Abdullah, M., Iqbal, M. S., Rana, R. M., Yasir, M., Khalid, S., Javed, T., and Mora-Poblete, F. (2021). Genetic potential and inheritance pattern of phenological growth and drought tolerance in cotton (Gossypium hirsutum L.). *Frontiers in Plant Science* **12**, 705392.

Mnookin, J. L. (2017). Fingerprint evidence in an age of DNA profiling. *In* "Expert Evidence and Scientific Proof in Criminal Trials", pp. 163-220. Routledge.

Niu, H., Ge, Q., Shang, H., and Yuan, Y. (2022). Inheritance, QTLs, and candidate genes of lint percentage in upland cotton. *Frontiers in Genetics* **13**, 855574.

Niu, X.-M., Xu, Y.-C., Li, Z.-W., Bian, Y.-T., Hou, X.-H., Chen, J.-F., Zou, Y.-P., Jiang, J., Wu, Q., and Ge, S. (2019). Transposable elements drive rapid phenotypic variation in Capsella rubella. *Proceedings of the National Academy of Sciences* **116**, 6908-6913.

Nyongesa, S. P. (2017). Field and molecular screening for striga resistance in selected finger millet (Eleusine coracana, L. Gaertn) germplasm in western Kenya, University of Eldoret.

Ormel, J., Hartman, C. A., and Snieder, H. (2019). The genetics of depression: successful genome-wide association studies introduce new challenges. *Translational psychiatry* **9**, 114.

Park, S.-H., Scheffler, J. A., Ray, J. D., and Scheffler, B. E. (2021). Identification of simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) that are associated with the nectariless trait of Gossypium hirsutum L. *Euphytica* **217**, 1-17.

Sabev, P., Valkova, N., and Todorovska, E. G. (2020). Molecular markers and their application in cotton breeding: progress and future perspectives. *Bulgarian Journal of Agricultural Science* **26**.

Salisu, I., Olawale, A., Jabbar, B., Koloko, B., Abdurrahaman, S., Amin, A., and Ali, Q. (2018). Molecular markers and their Potentials in Animal Breeding and Genetics. *Nigerian Journal of Animal Science* **20**, 29-48.

Shamshad, M., and Sharma, A. (2018). The usage of genomic selection strategy in plant breeding. *Next generation plant breeding* **26**, 93-108.

Shimizu, T., Aka Kacar, Y., Cristofani-Yaly, M., Curtolo, M., and Machado, M. A. (2020). Markers, maps, and marker-assisted selection. *The Citrus Genome*, 107-139.

Simair, A. A., and Simair, S. P. (2020). Status and recent progress in determining the genetic diversity and phylogeny of cotton crops. *Cotton Science and Processing Technology: Gene, Ginning, Garment and Green Recycling*, 15-37.

Suomela, J. A., Viljanen, M., Svedström, K., Wright, K., and Lipkin, S. (2023). Research methods for heritage cotton fibres: case studies from archaeological and historical finds in a Finnish context. *Heritage Science* **11**, 175.

Tarach, P. (2021). Application of polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) in the analysis of single nucleotide polymorphisms (SNPs). *Acta Universitatis Lodziensis. Folia Biologica et Oecologica* **17**, 48-53.

Ujjainkar, V. Genetic Improvement of Cotton through Molecular Breeding.

Ulloa, M., Hulse-Kemp, A. M., De Santiago, L. M., Stelly, D. M., and Burke, J. J. (2017). Insights into upland cotton (Gossypium hirsutum L.) genetic recombination based on 3 high-density single-nucleotide polymorphism and a consensus map developed independently with common parents. *Genomics Insights* **10**, 1178631017735104.

Viot, C. (2019). Domestication and varietal diversification of Old World cultivated cottons (Gossypium sp.) in the Antiquity. *Revue d'ethnoécologie*.

Waghmare, V. N. (2022). Cotton Breeding. *In* "Fundamentals of Field Crop Breeding", pp. 609-676. Springer.

Wahab, M. A. (2017a). Molecular diversity analysis and polymorphism study in cotton (Gossypium hirsutam L.) genotypes through rapd markers, Department of Biotechnology.

Wahab, M. A. (2017b). Through rapd markers.

Wallace, J. G., and Mitchell, S. E. (2017). Genotyping-by-sequencing. *Current Protocols in Plant Biology* **2**, 64-77.

Wang, J., Chen, Z., Jin, S., Hu, Z., Huang, Y., and Diao, Y. (2017). Development and characterization of simple sequence repeat (SSR) markers based on a full-length cDNA library of Napier Grass (Pennisetum purpureum Schum). *Genes & Genomics* **39**, 1297-1305.

Wu, Y., Chen, D., Zhu, S., Zhang, L., and Li, L. (2017). A new sythetic hybrid (A1D5) between Gossypium herbaceum and G. raimondii and its morphological, cytogenetic, molecular characterization. *Plos one* **12**, e0169833.

Yu, J., Jung, S., Cheng, C.-H., Lee, T., Zheng, P., Buble, K., Crabb, J., Humann, J., Hough, H., and Jones, D. (2021). CottonGen: The community database for cotton genomics, genetics, and breeding research. *Plants* **10**, 2805.

Zaki, H., and Hussein, N. R. (2023). Inter simple sequence repeats and morphological traits to identify cultivated cotton varieties (Gossypium barbadense L.) in Egypt. *Genetic Resources and Crop Evolution* **70**, 993-1006.

Zhang, T.-T., Zhang, N.-Y., Li, W., Zhou, X.-J., Pei, X.-Y., Liu, Y.-G., Ren, Z.-Y., He, K.-L., Zhang, W.-S., and Zhou, K.-H. (2020). Genetic structure, gene flow pattern, and association analysis of superior germplasm resources in domesticated upland cotton (Gossypium hirsutum L.). *Plant diversity* **42**, 189-197.

Zhang, Y., Zhang, Y., Ge, X., Yuan, Y., Jin, Y., Wang, Y., Zhao, L., Han, X., Hu, W., and Yang, L. (2023). Genome-wide association analysis reveals a novel pathway mediated by a dual-TIR domain protein for pathogen resistance in cotton. *Genome Biology* **24**, 111.

Declaration

Conflict of interest

There is no conflict of interest among the authors.

Data Availability statement

All authenticated data have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this paper.

Consent for publication

Not applicable

Funding

There were no sources providing support, for this paper.