



## IMPROVEMENT OF COTTON VARIETIES USING BIOTECHNOLOGICAL TOOLS: A REVIEW

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### ABSTRACT

Cotton is an important cash crop, contributes significantly to the agricultural economy, provides income to the farmers and creates numerous jobs in the textile and clothing industries. Cotton cultivation has many advantages, but it also has drawbacks, such as high-water use, pesticide use, and other environmental concerns. The goal is to reduce these problems and make cotton production more eco-friendly using sustainable farming techniques and crop technologies. To meet the ever-growing demands of the expanding population, crop development tactics have now all become reliant on biotechnological advancements. During last three decades, biotechnology has provided a huge range of opportunities, especially in the form of Molecular indicators that can help in identifying transgenic plants which can withstand biotic and abiotic challenges in the more expedient selection of parents and offspring in traditional breeding schemes intended to crop enhancement. This review aimed to acquaint with some fundamental elements, as well as the state and future of cotton biotech research.

**KEY WORDS:** Cotton, Biotechnology, Molecular indicators, Transgenic plants

### INTRODUCTION

Cotton is one of the most significant cash crops in the world's economy and has been farmed for a very long period (Lloyd May, 2002). It is regarded as the foundation of the textile industry and the most commercially significant fibre crop (Gulati *et al.*, 1929; Tausif *et al.*, 2018). Cotton production and marketing faces several challenges, including competition from synthetic fibre, yield fluctuations from year to year, and increased fibre quality standards brought about by technical advancements in the textile sector (Hayat *et al.*, 2020). Global cotton crop productivity is greatly reduced due to weeds, diseases, pests, and several other environmental factors. Cotton cultivars have been successfully produced to incorporate a number of beneficial agronomic traits, including early maturity, improved fibre quality, heat tolerance, etc., by utilising standard hybridization and mutation breeding (Bolek *et al.*, 2016). A few crop security strategies can assist in reducing notable crop losses brought on by biotic factors. Recent years have seen groundbreaking achievement made possible by adoption of modern biotechnology techniques (Wilkins *et al.*, 2000; Katageri *et al.*, 2021; Khan *et al.*, 2022).

Cotton plant belongs to the genus, *Gossypium*, family Malvaceae which produces the single most important textile fiber, accounting for ~35% of the world's total annual fiber demands (Fryxell, 1992). Cotton plant is also used as a model system to study plant polyploidization, cell elongation, and cell wall biogenesis (Yang *et al.*, 2020). The *Gossypium* genus comprised 45 diploid species ( $2n = 2x = 26$ ) and seven tetraploid species ( $2n = 4x = 52$ ) with extraordinary morphological variations, including different plant architectures ranging from wild perennial small trees and shrubs to cultivated herbaceous annuals, with variable leaf shapes and different fiber characteristics.

Among the several domesticated taxa of *Gossypium* in the Malvaceae family that are grown extensively, and four species (*Gossypium hirsutum*, *Gossypium barbadense*, *G. herbaceum*, and *Gossypium arboreum*) are cultivated for fiber production. Over 90% of cotton produced in the world comes from Upland cotton (*Gossypium hirsutum*) (Wang *et al.*, 2019). The genetic improvement of cotton plant has been ongoing for centuries, but recent advances in genetic engineering and genomics have made this process faster and more efficient (Fernandez-Cornejo *et al.*, 2002). Due to the development

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of strong transformation and regeneration methods (Flachs, 2017), cotton has been the crop most frequently modified by genetic engineering tools (Chakravarthy *et al.*, 2014; Tarazi *et al.*, 2019). It also contains the most recent information on the genetic improvement of cotton through biotechnological interventions for the improvement of cotton fibre and the control of biotic (disease resistance, etc.) and abiotic (drought, climate resilience, etc.) stresses (Kamburova *et al.*, 2022) and the contribution of epigenetics in increasing cotton fibre productivity and quality has also been addressed (Kohel *et al.*, 2001).

Biotechnological tools have been increasingly employed to enhance the improvement of cotton varieties, aiming to achieve traits such as increased yield, resistance to pests and diseases, improved fiber quality, and tolerance to environmental stress. Some key biotechnological tools and techniques utilized in the improvement of cotton varieties include:

#### **Genetic Engineering (Genetic Modification):**

Genetic engineering has provided mankind with unprecedented power to manipulate and develop novel crops towards a safe and sustainable agriculture in the 21<sup>st</sup> century (Gilbert, 2013; Arora *et al.*, 2017). Technologies and chemical interventions that have proven harmful to human health and environment need to be replaced with safer alternatives to manage insect pests in agricultural ecosystems (Ahmad *et al.*, 2017). Many insecticidal proteins (Debbie *et al.*, 1996) and molecules are available in nature which are effective against agriculturally important pests but are innocuous to mammals, beneficial insects, and other organisms. Insecticidal proteins present in *Bacillus thuringiensis* (Bt), which have shown efficacy as spray formulations in agriculture over the past five decades, have been expressed in many crop species with desirable outcome. Bt technology is reported to be effective against chewing larvae of orders lepidoptera, coleoptera, and diptera but not for sap-sucking pests of order Hemiptera (Palma *et al.*, 2014; Paul & Das, 2021). Next generation transgenics have also been developed by stacking multiple insecticidal genes. Coexpression of two cry genes (Cry1Ac and Cry2Ab) into the crop imparted defense against a wider range of pests and was commercialized in 1999 with a big success (Sivasupramaniam *et al.*, 2014). It was highly toxic to *H. armigera* and two species of armyworms *S. frugiperda* and *S. exigua* compared to cotton expressing Cry1Ac (Stewart *et al.*, 2001). These insecticidal proteins are highly specific to their target pests and are proven to be biodegradable and safe for mammals and other non-target organisms (Federici, 2003; Oliveira *et al.*, 2007). Large scale cultivation of Bt-crops raises concerns about the possible

development of resistance in insects. Many strategies have been formulated to prevent/delay the development of resistance.

#### **RNA Interference (RNAi):**

RNAi approaches for pest control that do not include transgenic application include soil treatment, foliar spraying, immersing leaf tissues in dsRNA solution, and stem injections (Cagliari *et al.*, 2019, Dalakouras *et al.*, 2020). The RNAi approach effectively mutes the genes in a short period without causing heritable alterations to the genome and have a higher public acceptability. Spraying of dsRNAs to regulate pest populations is a safe and eco-friendly approach, and dsRNA has short residue period, therefore, it has huge potential for adoption by plant growers (Cagliari *et al.*, 2019). RNAi for the control of *B. tabaci* through oral delivery of dsRNA/siRNA was demonstrated (Upadhyay *et al.*, 2011) and its potential was further validated in transgenic tobacco as proof of concept for field applications (Thakur *et al.*, 2014, Grover *et al.*, 2019).

#### **Marker-Assisted Breeding (MAB):**

Cotton genetic resources contain diverse economically important traits that can be used widely in breeding approaches to create of high-yielding elite cultivars with superior fiber quality and adapted to biotic and abiotic stresses. Molecular markers gave a great opportunity to improve the efficiency and precision of crop improvement programs via marker-assisted selection (Collard & Mackill, 2008). The use of DNA markers in plant breeding is called marker-assisted selection (MAS) and it is a component of the molecular breeding approach (Collard & Mackill, 2008). MAS technology allows conducting the selection at any stage of plant growth and development. In short, the development of the MAS technology aimed at the selection of crops led to high achievements in genomics, which became a vital part of agricultural science.

Marker-assisted breeding involves the use of DNA markers associated with desirable traits to accelerate the traditional breeding process (Fakhriddin *et al.*, 2020). This allows breeders to select plants with specific traits more efficiently, such as disease resistance or improved fiber quality.

#### **Genomic Selection:**

Whole Genome Sequencing: Advances in genomics enable the sequencing of the entire cotton genome, allowing breeders to identify specific genes associated with desirable traits (Grover *et al.*, 2004). Genomic selection involves predicting the performance of a plant based on its genomic profile, accelerating the breeding process (Char *et al.*, 2017).

### Gene Editing:

The past few years have seen, rise in the accessibility and significance of genome editing tools for agricultural production (Gao *et al.*, 2017). Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated (Cas) systems are the three main tools for targeted mutagenesis (Gilbert *et al.*, 2014). These tools can all be combined to create extremely specific double-strand breaks in chromosomal DNA (Konermann *et al.*, 2015). Localised mutations and deliberate sequence replacements result from the processing of these breaks by homologous recombination (HR) and nonhomologous end joining (NHEJ) repair mechanisms. The CRISPR/Cas system is high-efficient in producing targeted mutagenesis and is simpler to utilise than the ZFN and TALEN systems (Gao *et al.*, 2017; Wang *et al.*, 2019).

### Crispr-Cas9 Technology:

A more focused, effective, and rapid GE technique CRISPR/Cas9 (Khatodia *et al.*, 2016) has been successfully used to modify the genomes of many different creatures, including humans. The technique is continuously being improved for its use in both prokaryotes and eukaryotes. Advancements in this methodology have enabled the achievement of high-throughput screening for genomic gene identification (Tsai *et al.*, 2016). The cotton species *G. hirsutum* is the most broadly cultivated, but it is susceptible to various biotic stress which seriously limit fiber yield and quality (Briddon *et al.*, 2000). Genome editing has transformed the agricultural landscape with many success stories of engineering biotic stress tolerance in crop plants (Grohmann *et al.*, 2019). The tremendous potential of genome editing to improve crop plants has been demonstrated using model systems for various traits such as biotic and abiotic stress tolerance, nutritional enhancement, and yield improvement (Kim *et al.*, 2014). In prokaryotes, CRISPR/Cas9 was originally discovered as a natural defence mechanism against viral attacks (Bhaya *et al.*, 2014). CRISPR/Cas has been proved effective for the targeting and redesigning of crop genomes (Doudna *et al.*, 2014), especially crop like cotton which are difficult to manipulate with conventional genetic engineering (Xu *et al.*, 2016). Cas9 nuclease has been characterized with two catalytic domains (HNH and RuvC) for creating double-strand breaks (DSBs) in the targeted genomic site. The specificity of CRISPR/Cas9 system to create DSBs is driven by two RNA molecules; trans-activating crRNA (tracrRNA) and CRISPR RNA (crRNA) and a protospacer adjacent motif (PAM) necessary for recognition of target site in the genome (Ma *et al.*, 2015). Although, the CRISPR/Cas9 system has been the most extensively used genome

editing system by the scientific community, in recent years, several new CRISPR/Cas variants such as CRISPR/Cas12, CRISPR/CasX, CRISPR/Cas13, CRISPR/Cas3, and CRISPR/Cas14 have been discovered with different PAM requirements, smaller Cas proteins, and improved cleavage efficiency. CRISPR/Cas9, CRISPR/Cas12, and CRISPR/Cas13 have been successfully used for genetic improvement of plants of desired traits (Ma *et al.*, 2015; Ji *et al.*, 2015).

### Omics Technologies:

Proteomics, Metabolomics, and Transcriptomics: These technologies enable a comprehensive understanding of the molecular processes within cotton plants. By studying the proteins, metabolites, and gene expression patterns, researchers can identify key pathways and target for improvement. Next-generation sequencing (NGS) has significantly increased sequencing efficiency and reduced cost due to the quick development of sequencing technology. This has led to the generation and distribution of enormous volumes of genomic, transcriptomic, and epigenetic data pertaining to cotton species. The study of population genetic diversity, cotton genomic structure and evolution, and the extensive mining of the genome for exceptional genes pertaining to significant traits are all made possible by these massive data sets. The CottonGen (Yu *et al.*, 2014) provides comprehensive information for cotton genomes, genetic markers, breeding cultivar phenotypes, etc., but its searching and visualization interface needs to be improved; the ccNet (You *et al.*, 2017) focuses mainly on co-expression networks of tetraploid *G. hirsutum* and diploid *G. arboreum*; the CottonFGD (Zhu *et al.*, 2017) is a cotton functional genome database that integrates cotton genomes, transcriptome data and a variety of online tools, but it lacks information for genetic resources; the MaGenDB (Wang *et al.*, 2019) is a comprehensive database for 13 Malvaceae species including cotton, so it is convenient to perform genetic comparative analyses among Malvaceae species; however, its functional analyses on cotton are not complete. Established for cotton research, COTTONOMICS is a dependable platform that combines genetic information, multi-omics data, and integrated genomic resources (Dai *et al.*, 2022).

### Bioinformatics:

Bioinformatics plays a crucial role in analysing large-scale genomic and omics data. It helps in the identification of potential genes, the comprehension of gene function, and the forecasting of the consequences of genetic alterations. The sequential integration of individual omics data and its comprehension of the interactions between different cellular molecule types and their roles to enhance

resilience to biotic and abiotic stresses and raise lint yield and quality are made possible by bioinformatics. Moreover, multi omics-integrated informatics lend a helping hand in assessing the information flow from one level of omics to another and consequently help in interrelating genotype to phenotype (Aslam, 2022).

### Synthetic Biology: Pathway Engineering:

Synthetic biology approaches involve the design and construction of new biological pathways. This can be applied to engineer cotton plants for improved traits, such as enhanced fiber production or resistance to specific stresses.

The combination of these biotechnological tools provides a powerful toolbox for cotton breeders to develop improved varieties that meet the demands of sustainable agriculture, ensuring better yields, reduced environmental impact, and increased resilience to changing climatic conditions (Wen *et al.*, 2023). Additionally, regulatory frameworks and public acceptance are crucial considerations in the deployment of biotechnological advancements in agriculture (Singh, 2021).

### CONCLUSION

Despite cotton's susceptibility to variety of illnesses and pests, cotton production relies on a broad range of biotechnological approaches to reduce losses. These biotechnological innovations reduce production costs and provide market and global manufacturing with environmental friendly control systems (Verma *et al.*, 2023). Ag biotech cotton is being used in production areas of the main cotton-producing countries and is gaining trust on a worldwide scale (Geoffery *et al.*, 2014). Continuous development in genome editing and sequencing technologies for cotton, along with the improvement of transgenic technology and marker-assisted selection, will surely lead to the introduction of new stress-tolerant and stress-resistant cotton germplasms in the upcoming years. By utilising these novel resistant germplasms along with enhanced bioagents, phytopathogen sanitation techniques can be completely transformed, leading to a decrease in losses and an improvement in environmental conditions (Herman *et al.*, 2019).

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