Journal of Cotton Research

REVIEW

Open Access

CRISPR/Cas genome editing for cotton precision breeding: mechanisms, advances, and prospects

SHERI Vijay[1], MOHAN Harikrishnan[2], JOGAM Phanikanth[3], ALOK Anshu[4], ROHELA Gulab Khan[5] and ZHANG Baohong[1*]

Abstract

Cotton (*Gossypium hirsutum* L.) is one of the most important global crops that supports the textile industry and provides a living for millions of farmers. The constantly increasing demand needs a significant rise in cotton production. Genome editing technology, specifically with clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) tools, has opened new possibilities for trait development in cotton. It allows precise and efficient manipulation within the cotton genome when compared with other genetic engineering tools. Current developments in CRISPR/Cas technology, including prime editing, base editing, and multiplexing editing, have expanded the scope of traits in cotton breeding that can be targeted. CRISPR/Cas genome editing has been employed to generate effectively CRISPRized cotton plants with enhanced agronomic traits, including fiber yield and quality, oil improvement, stress resistance, and enhanced nutrition. Here we summarized the various target genes within the cotton genome which have been successfully altered with CRISPR/Cas tools. However, some challenges remain, cotton is tetraploid genome having redundant gene sets and homologs making challenges for genome editing. To ensure specificity and avoiding off-target effects, we need to optimize various parameters such as target site, guide RNA design, and choosing right Cas variants. We outline the future prospects of CRISPR/Cas in cotton breeding, suggesting areas for further research and innovation. A combination of speed breeding and CRISPR/Cas might be useful for fastening trait development in cotton. The potentials to create customized cotton cultivars with enhanced traits to meet the higher demands for the agriculture and textile industry.

Keywords CRISPR/Cas, Biotic stress, Fiber quality, Genomic complexity, Off-target effects, Textile industry

Introduction

Cotton is seed-hair fiber and a very significant source for natural fiber, oil, and livestock feed (Kumar et al. 2023; Xu et al. 2024). Cotton genome has different ploidy types and complex structure; cultivated commercial cotton are allotetraploids (AD) species, in which *Gossypium hirsutum* (upland cotton) has a genome size of 2.5 Gb (Peng et al. 2021). Cotton fibers consist of about 87%–90% cellulose, a carbohydrate derived from plants, along with 5%–8% water and 4%–6% naturally occurring impurities (Prabhu et al. 2012). These characteristics enable cotton to withstand high pressing temperatures, accept a wide

*Correspondence:
Zhang Baohong
zhangb@ecu.edu
[1] Department of Biology, East Carolina University, Greenville, NC 27858, USA
[2] Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA
[3] Department of Biotechnology, Kakatiya University, Warangal, Telangana 506009, India
[4] Department of Plant Pathology, University of Minnesota, Saint Paul, MN 55108, USA
[5] Central Sericultural Research & Training Institute, Central Silk Board, Jammu & Kashmir, Pampore 192121, India

range of dyes, and remain washable (Todor et al. 2021). The seeds of cotton are high in protein and oils, making them suitable for the production of oil, animal feed, and industrial items like soaps and cosmetics (Pan et al. 2020). Because of its valuable oilseeds and fibers for the food and textile sectors, cotton has become highly recognized as an important cash crop and a good source of biofuels (Oliveira et al. 2016). The fibers can be treated to create a wide variety of textiles, such as lightweight voiles and laces, thick-piled velveteen, and heavy sailcloth, which are perfect for a variety of industrial uses as well as home furnishings and apparel applications (Wilson 2011). Cotton-based fabrics are known for their high abrasion resistance and durability, with their primary component being cellulose, which forms the structural framework of cotton fiber (Dochia et al. 2012). Its ability to absorb and release moisture quickly also makes it a comfortable fabric for clothing. Cotton is versatile, used in various applications such as bandages, tablecloths, single-use clothing and bedding in hospitals, and other healthcare facilities (Morris et al. 2020).

Cotton can be cultivated majorly between the latitudes of 30° N and 30° S, where weather conditions have a substantial impact on the quality and production of fiber (Shuli et al. 2018). The different kinds of cotton that are farmed as crops are indigenous to the majority of the world's subtropical regions and have undergone multiple independent domestications. In temperate regions, cotton is typically grown as a shrubby annual, but it can also be found as perennial trees in tropical climates (Singh et al. 2007). Although it can reach heights of up to 6 m in tropical climates, its typical height under cultivation varies from 1 to 2 m (Hussain et al. 2020). The plant produces white blossoms within 80–100 d of sowing, which eventually become crimson in color.

Numerous insect species attack cotton plants, which include dangerous ones like the boll weevil, conchuela, aphid, cotton flea hopper, cotton leaf worm, grasshoppers, pink bollworm, rapid plant bug, spider mites (red spiders), southern green stinkbug, tarnished plant bugs, and thrips (Kiobia et al. 2023). By carefully choosing varieties with some resistance to insect damage, as well as other cultural techniques like planting at the right time, it is possible to control the damage caused by insect pests to a limited extent. Due to ecological concerns, chemical pesticides, which were originally developed in the early 1900s, must be used carefully and selectively, as they can harm the beneficial non-target organisms (Samada et al. 2020).

Studying functional genomes in cotton have been significantly aided by current advancements in genome sequencing (Zhang et al. 2015; Yuan et al. 2015; Li et al. 2015). The urgent need for quick and affordable techniques to make targeted mutations in cotton has been highlighted by the increase in the availability of sequences (Long et al. 2018; Li et al. 2015; Wang et al. 2016, Zhang et al. 2015). Numerous success stories of genetically modifying crop plants to withstand biotic stress have changed the face of agriculture. Model systems for diverse features, that include modification and enhancement of genes response for nutritional enhancement, abiotic and biotic stress tolerance, and in addition to yield and quality improvement, have been used to illustrate the enormous possibilities for genome editing to advance crop plants.

Recent advances in genome editing approaches, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), as well as clustered regularly interspaced short palindromic repeats (CRISPR/CRISPR associated protein (Cas), such as CRISPR/Cas9, changed plant research by allowing precise gene modification (Li et al. 2021b, Li et al. 2024a Sandhya et al. 2020; Jogam et al. 2022; Saeed et al. 2023). Among these innovations, CRISPR/Cas9 stands out for its robustness and ease of use. This method consists of two key components: (1) Cas9 enzyme nuclease, which causes double- strand breaks in DNA, and (2) a single guide RNA (sgRNA) guiding Cas9 to the desired target region (Sandhya et al. 2020). Currently, CRISPR/Cas9 is considered as a promising method for introducing precise modifications in the genome of different plant species including the cotton (Hsu et al. 2014; Yang et al. 2015; Doudna et al. 2014; Bassett et al. 2013; Li et al. 2021a; Li et al. 2024a). Although cotton functional genomics research is still somewhat behind that of model plant species, such as *Arabidopsis* and rice, CRISPR/Cas9 and CRISPR/Cas12a (Cpf1) systems have been used to edit cotton genomes (Li et al. 2017; Wang et al. 2018; Chen et al. 2017; Li et al. 2019a, b, c). Further, the limits of conventional resistance breeding can potentially be addressed to a great extent by advances in genome editing technology. Plant scientists have effectively used CRISPR/Cas-based gene editing to improve the cotton traits (Fig. 1), such as fiber, oil content, abiotic and biotic stress tolerance (Table 1). This review thoroughly discusses all the most recent breakthroughs and improvements made in relation to genome editing in cotton.

Targeted genes and their editing efficiency within cotton genome

One of the significant difficulties in cotton genome modification is its complex allotetraploid genome. This genome contains A and D subgenomes, each containing multiple copies of many genes with high sequence homology (Li et al. 2015). The efficiency of gene editing (Table 2) by CRISPR/Cas9 mainly depends on the accuracy of sgRNA, designed with the aid of computational

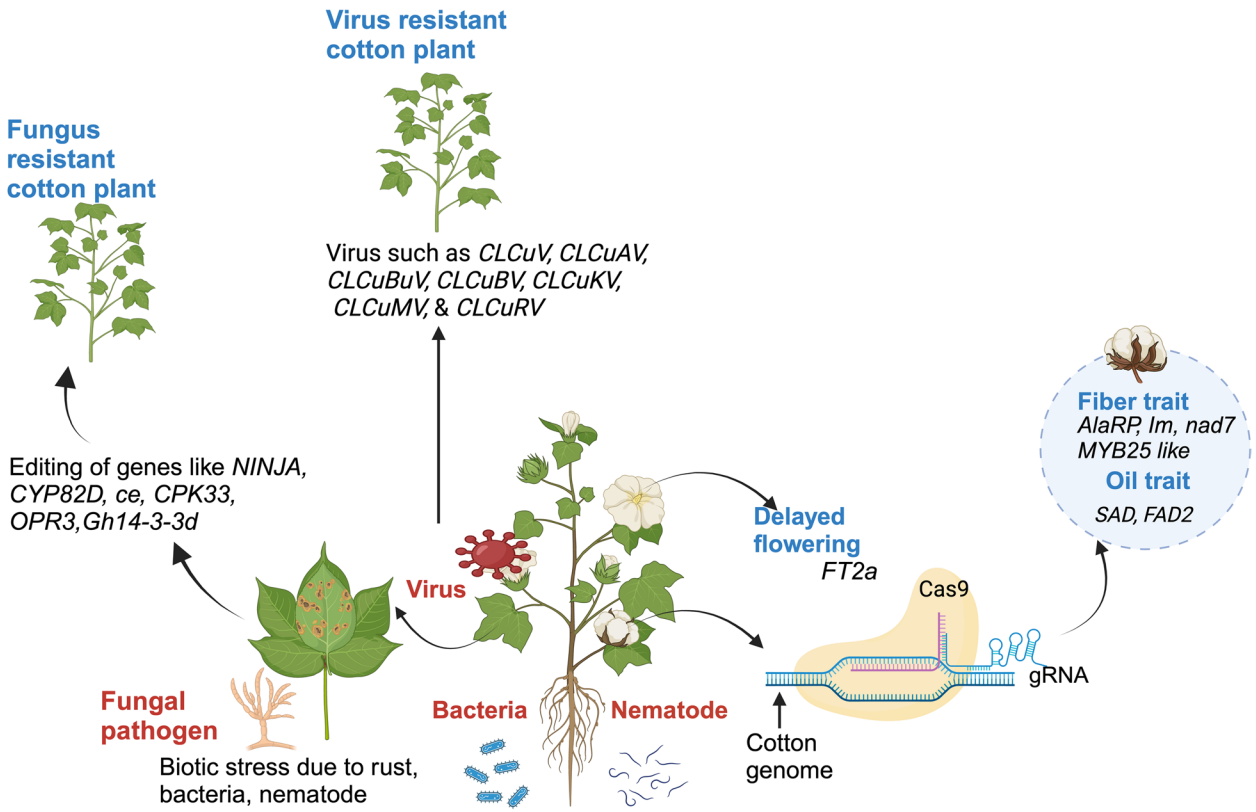

Fig. 1 Various traits improved in cotton through genome editing by targeting specific genes with the use of CRISPR/Cas to develop tolerance to abiotic and biotic stresses, as well as enhanced fiber and oil yield

algorithms (Li et al. 2023). These algorithms help ensure sgRNA directs the Cas9 protein to the correct location in the genome, improving the accuracy of genetic alterations. Not all of them, though, are efficient in all crops, thus it's critical to have a quick and flexible validation technique to ascertain the gRNA's efficacy (Gao et al. 2017; Li et al. 2019d; Long et al. 2018). The first report of CRISPR/Cas mediated genome editing in cotton was published by Li et al. (2017). They employed CRISPR/Cas9 system and successfully knocked out *GhMYB25-like A* and *GhMYB25-like D* gene with 14.2%–21.4% fragment termination events at the target sites. The frequency of mutations at the *GhMYB25-like A* and *GhMYB25-like D* DNA loci were found to be 100% and 98.8%, respectively, based on the PCR product sequencing results. In this study they have concluded that the CRISPR/Cas9 technique may be a useful strategy for targeted mutagenesis in cotton genome by emphasizing that off-target induced mutation occurrences have not been discovered in their transgenic plants, even for a gene only have mismatch with sgRNAs (Li et al. 2019b). Gao et al. (2017) targeted *GhEF1* and *GhPDS* genes using two sgRNA expression cassettes as well as two sgRNAs within *GhPDS* gene for fragment deletion. Chen et al. (2017) developed specific gRNAs to target *GhCLA*1 and *GhVP* genes and validated the efficacy of the CRISPR/Cas9 system in cotton using protoplasts. The mutations in the transfected protoplast cells were analyzed by the restriction enzyme (RE)-PCR assay as the alterations in the target site abolished the restriction enzyme recognition sites. Further sequencing results confirmed the mutations in target genes and most of them are nucleotide substitutions. Stable transformation using *Agrobacterium* generated edited plants with 47.6%–81.8% efficient mutations. Molecular investigation of the transgenic cotton plants utilizing an reverse transcription (RT)-PCR test and sequencing further confirmed the mutations and also detected no off-target alterations (Chen et al. 2017). Long et al. (2018) also settled a new system to test the efficacy of CRISPR/Cas9 genome editing system in cotton plants using transient assay by incorporating the endogenous GhU6 promoter for expressing gRNA instead of the AtU6 promoter. The results displayed the expression levels of sgRNAs were 6–7 times higher when expressed under the control of endogenous GhU6 promoter compared with AtU6 promoter as well as the mutation efficiency was 4–6 times higher. Li et al. (2017) created two sgRNAs, GhMYB25-like-sgRNA1 along with GhMYB25-like-sgRNA2, within

Table 1 Recent advancement in cotton via CRISPR/Cas based genome editing

S. No	Targeted gene	Type of nuclease	Outcome	Reference
Fiber development and yield increase				
1	*GhHDZ76*	Cas9	Fiber development	Wu et al. 2024
2	*GhWER*	Cas9	Fiber initiation and epidermal development	Zhao et al. 2024
3	*GhMYB52*	Cas9	Enhances lint yield	Yang et al. 2024
4	*GhFAD2*	Cas9	Improved the quality of seed oil	Chen et al. 2021
5	*GhMYB25-like*	Cas9	Key factor in early cotton fibre development	Li et al. 2017
6	*GhPDCT*	Cas9	Increased oleic acid	Li et al. 2024a
7	*GoPGF*	Cas9	Reduces gland density	Janga et al. 2019
Physiological changes and architecture variation				
8	*GhCLA1*	Cas9	Albino development	Gao et al. 2017
9	*GhCLA*	Cas12a	Albino development	Li et al. 2019a
10	*GHCU*	Cas9	Abnormal leaf shape	Zang et al. 2024
11	*GhTFL1*	nCas9	Architecture variation	Wang et al. 2024
12	*GhARG*	Cas9	Increased lateral root formation	Wang et al. 2017
13	*Gh4CL20/24A*	Cas9	Reduced flavonoid content	Gong et al. 2024
14	*GhALARP*	Cas9	Preferentially expressed in cotton fibers	Zhu et al. 2018
15	*GhPGF* and *GhRCD1*	Cas9	Efficient callus proliferation	Ge et al. 2023
Gossypol elimination				
16	*GhDIR5*	Cas9	Elimination of gossypol	Lin et al. 2023
17	*GhPGF*	Cas12a	Elimination of gossypol	Li et al. 2021a
Male sterility induction				
18	*GhAOC2*	Cas9	Development of male sterility	Khan et al. 2023b
19	*GhEMS1*	Cas9	Male sterility development	Zhang et al. 2023
20	*GhDMP*	Cas9	Maternal haploid induction	Long et al. 2024
Abiotic and biotic resistance				
21	*Gh14-3-3d*	Cas9	Resistance to *Verticillium dahliae*	Zhang et al. 2018b
22	*GhMIR482*	Cas9	*Verticillium dahliae* disease resistance	Zhu et al. 2022
23	*GhRCD1*	Cas9	Reduced cadmium resistance	Wei et al. 2024
24	*GhTULP34*	dCas9	Drought tolerant	Yu et al. 2023

identical genomic regions of *GhMYB25-like A* and *GhMYB25-like D*, for directing Cas9-mediated allotetraploid cotton genome modification. According to their findings, a high proportion (14.2%−21.4%) of CRISPR/Cas9-induced deletions from either of the indicated DNA locations were discovered. Additionally, their sequencing results showed that the target locations had 100% and 98.8% mutation frequency, respectively, with no evidence of off-target mutations. These findings demonstrate that CRISPR/Cas9 is one of the most effective strategies for producing high efficiency and specificity DNA level alterations on the allotetraploid cotton genome.

To target various genomic locations simultaneously in the allotetraploid cotton genome, Wang et al. (2018) designed multiple sgRNAs targeting exogenously transformed *DsRed2* and the native gene *GhCLA1*. The results demonstrated that *DsRed2* edited T_0 generation plants reverted to its wild type phenotype with inheritability to subsequent generations. On the other hand, 75% of the T_0 plants exhibited an albino phenotype for the endogenous target gene *GhCLA1*, with effective alterations seen at target locations. High throughput barcode-based sequencing showed no off-target modifications.

Programmable site-specific nucleases have the ability to cause double-strand breakage in the target region, which can lead to exact DNA sequence substitution by homology-directed repair (HDR) or mutations by an error prone non-homologous end joining (NHEJ) repair mechanism. However, HDR is highly inefficient in plants due to its complexity and delivery of the repair templates, which has greatly limited precision genome editing. Hence, it is required to establish an alternate strategy for precision editing. Prime editing and base editing are emerging precision editing techniques based on the CRISPR/Cas system. Qin et al. (2020) employed base editing to edit a gene in the allotetraploid cotton genome; at three target locations, the base editing efficiency ranges from 26.67% to 57.78%. Further, deep sequencing

Table 2 Targeted genes and their editing efficiency within the cotton genome

S. No	Targeted gene	Type of nuclease	Editing efficiency /%	Reference
1	*GhMYB25-like A*	Cas9	100.0	Li et al. 2017
2	*GhMYB25-like D*	Cas9	98.8	Li et al. 2017
3	*GhCLA*1	Cas9	47.6	Chen et al. 2017
4	*GhVP*	Cas9	81.8	Chen et al. 2017
5	*GhAlaRP-A*	Cas9	71.4–100.0	Zhu et al. 2021
6	*GhAlaRP-D*	Cas9	92.9–100.0	Zhu et al. 2021
7	*GhCLA*	nCas9	26.67	Qin et al. 2020
8	*GhPEBP*	nCas9	27.50	Qin et al. 2020
9	*GhCLA1*	Cas9	90.0	Li et al. 2022
10	*GhAOC2*	Cas9	80.0	Khan et al. 2023b
11	*GhCLA1*	Cas9	80.6	Gao et al. 2017
12	*GhPDS*	Cas9	28.26–55.43	Lei et al. 2022
13	*GhCLA1*	Cas12a	87.0	Li et al. 2019a
14	*GhCLA1*	Cas9	66.7–100.0	Wang et al. 2017
15	*GhCLA*	Cas12b	6.34–98.68	Wang et al. 2020

studies showing C to T replacement in a specific editing window, spanning about −17 to −12 base pairs later the protospacer adjacent motif (PAM) sequence, which reached up to 18.63% of the overall sequences with no detectable off-target mutation at 1 500 predicted potential sites (Qin et al. 2020).

Though there have been lots of reports available about genome editing of cotton plants through CRISPR/Cas approach, a study explains the advantage of generating a founder transformants with integrated CRISPR/Cas that can be used as a baseline for targeting multiple sites by only expressing gRNAs (Aslam et al. 2022). Researchers introduced *DS-Red*, *Rep*, *Rec*, and CRISPR/Cas9 expressing constructs into cotton plants through genetic transformation. *DS-Red* was used as a visual marker to track the presence of the constructs (Sun et al. 2018). *Rec* and *Rep* genes were introduced to facilitate recombinase-mediated gene stacking, which allows for the precise integration and removal of genes of interest. The CRISPR/Cas9 system was utilized to produce targeted mutations or edits in specific genes. The development of these founder transformants serves as a starting point for further research on recombinase-mediated gene stacking in cotton. The capability to precisely stack the genes of interest in cotton genome has the potential to enhance desired traits and improve crop performance.

The application of gene editing and high-throughput whole genome sequencing in cotton is discussed in a forum article (Peng et al. 2021). In the article, the use of genome editing techniques in cotton was discussed, and genes linked to important agronomic traits specifically, fiber quality, yield, biotic and abiotic stress tolerance were identified. The article listed regulatory issues and off-target effects as well as other challenges associated with cotton genome editing (Peng et al. 2021).

Application of CRISPR technology for trait development in cotton

Genome modification for fiber improvements

Fiber length is a crucial quality parameter in cotton fibers, with transcriptomic studies identifying genes associated with fiber length that are predominantly expressed throughout the elongation phase of cotton fiber formation (Lee et al. 2007; Fang et al. 2024; Wu et al. 2024; Zhao et al. 2024; Yang et al. 2024). However, only some of these genes were functionally characterized. A study by Zhu et al. (2018) addressed this gap by developing a simplified CRISPR/Cas9 system to generate targeted alterations in the *GhAlaRP* gene, which translates an alanine-rich protein predominantly expressed in fibers of cotton during the elongation phase. The results demonstrated high efficiency in gene editing in upland cotton, with successful mutations at the intended target sites observed in 71.4%–100% of *GhAlaRP-A* and 92.9%–100% of *GhAlaRP-D* cases. Deletion events were the most common, with some instances of deletion accompanied by larger insertions. The majority of the transgenic plants demonstrated mosaic mutation activity, with no apparent off-target changes. This study generated mutant cotton plants that can be utilized to do future research into the role of *GhAlaRP* in fiber development. The relationship with fiber elongation has been verified using expression pattern analysis in growing fibers. Functional studies using gene disruption and modulation approaches such as RNA interference or CRISPR/Cas9-mediated gene editing have provided insight into the effect of *GhAlaRP*

on fiber elongation. These experiments suggest that *GhAlaRP* influences the fiber elongation process by regulating cell expansion and wall biosynthesis in cotton fibers. The encoded protein might interact with other cellular components involved in fiber elongation, potentially impacting cell wall properties and overall fiber quality (Zhu et al. 2021).

A study conducted by. Zhang et al. (2021a) through map-based cloning strategy and CRISPR/Cas9 gene editing technique cloned and analyzed the function of *GhIm* gene, which encodes a PPR protein associated with nonfluffy fiber phenotype. Specifically, this study investigated the involvement of *GhIm* in the splicing of mitochondrial *nad7* mRNA, which is crucial for normal mitochondrial function. The results demonstrated that reduced expression of *GhIm* and *nad7* led to defective fiber development, characterized by shorter fibers with irregular shapes and reduced cellulose content. Further analysis revealed that the downregulation of *GhIm* impaired the splicing of *nad7* mRNA, leading to mitochondrial dysfunction in cotton fibers. The study emphasizes the critical role of *GhIm* in the splicing of mitochondrial *nad7* mRNA during cotton fiber development. By ensuring proper splicing, *GhIm* contributes to the normal functioning of mitochondria, which is essential for fiber elongation and quality. This research provides vital insights into improving fiber yield and quality in cotton breeding programs.

Genome modification for oil improvements

In terms of edible oilseeds worldwide, cotton is ranked third behind soybean and canola oil. The main emphasis in genetic improvement of cotton has been on enhancing the yield and quality of its fiber, though there is an increasing demand for enhanced oilseed traits with high fatty acid content for biofuel applications (Wu et al. 2022; Shang et al. 2017; Chen et al. 2021; Zhang et al. 2021c; Li et al. 2024a). Cottonseed oil composition is primarily determined by the presence and activity of desaturase enzymes that are encoded by the *SAD* gene family. These enzymes facilitate the transformation of oleic acid (18:1) into stearic acid (18:0), a crucial step in the biosynthesis of monounsaturated fatty acids in cottonseed oil. To identify candidate genes within the *SAD* gene family, Shang et al. (2017) employed a combination of bioinformatic analyses and experimental validation to classify 9, 9, 18, and 19 *SAD* genes in the genomes of the four sequenced cotton species: diploid *G. arboreum* (A_2), G. *raimondii* (D_5), tetraploid *G. hirsutum* acc. TM-1 (AD_1), and *G. barbadense* cv. Xinhai 21 (AD_2), respectively. The researchers then conducted expression profiling of the identified candidate genes in various cotton plant tissues and developmental stages, with a particular focus on the developing seeds. This analysis helped determine the tissue-specific expression patterns of the genes and their potential role in cottonseed oil composition. Furthermore, functional validation experiments were carried out to estimate the impact of manipulating the expression of candidate genes on cottonseed oil composition. These experiments involved techniques such as gene silencing or overexpression in cotton plants, followed by analysis of the resulting changes in fatty acid profiles. Through this comprehensive approach, the study identified several candidate genes from the *SAD* gene family that are likely to play an important role in determining cottonseed oil composition. These genes provide potential targets for future genetic engineering or breeding strategies aimed at enhancing the industrial and nutritional qualities of cottonseed oil (Shang et al. 2017).

A recent review (Wu et al. 2022) explores various approaches aimed at enhancing the quality and nutritional value of cottonseed oil and protein. The review encompasses advancements in the realms of genetics, breeding, and genetic engineering that possess the ability to positively impact cottonseed traits. However, certain inherent limitations and undesirable characteristics have hindered the widespread utilization of cottonseed products.

Chen et al. (2021) used the CRISPR/Cas9 gene editing technology to precisely target and disrupt the *GhFAD2* gene in allotetraploid cotton. The *GhFAD2* gene encodes for a desaturase enzyme that translates oleic acid to linoleic acid, which is a detrimental trans-fatty acid due to its less oxidative stability. To increase the oleic acid content (stable and healthy fatty acid) in the cotton plant, the authors designed specific gRNAs to simultaneously target multiple copies of the *GhFAD2* gene family. The subsequent investigation validated the effective modification of these genes in cotton plants. To assess the impact of knocking out *GhFAD2* on cottonseed oil composition, the researchers conducted fatty acid profiling on the edited plants. They found a significant rise in the amount of oleic acid content and a fall in the amount of linoleic acid, resulting in a higher ratio of oleic acid to linoleic acid. This alteration makes the cottonseed oil more suitable for various industrial applications, including cooking, frying, and biodiesel production. One notable aspect of this approach is that it did not involve introducing foreign genetic materials or transgenes into the cotton plants, rendering them non-transgenic. This non-transgenic status is significant, as it can influence regulatory approval processes and public acceptance of gene-edited crops (Chen et al. 2021).

Stress resistant development using CRISPR tools

Cotton plants are continually subjected to both abiotic and biotic stressors, which significantly impact their growth, development and ultimately reducing the potential yield of cotton fibers (Aini et al. 2022; Ahmed et al. 2024; Sheri et al. 2023; Prakash et al. 2023; Umer et al. 2023; Gupta et al. 2024). Cotton production has declined in major cotton-growing regions, mostly owing to pest and disease pressures, and the application of management strategies. Among the primary factors impacting cotton yield worldwide is cotton leaf curl disease (CLCuD), which is triggered by cotton leaf curl virus (*CLCuV*) (Iqbal et al. 2016). Over the past 15 years, the Indian subcontinent has experienced a continuous threat to cotton production from different types of CLCuD, which is brought on by a variety of begomo-viruses, including the cotton leaf curl Alabad virus (*CLCuAV*), cotton leaf curl Bangalore virus (*CLCuBV*), cotton leaf curl Burewala virus (*CLCuBuV*), cotton leaf curl Kokhram virus (*CLCuKV*), cotton leaf curl Multan virus (*CLCuMV*), and cotton leaf curl Rajasthan virus (*CLCuRV*). In addition to vein enlargement and the formation of leaf-like, cup-shaped extensions (enations) on the bottoms of leaves, CLCuD causes stunted cotton plant development (Ahmad et al. 2011; Ali et al. 2013; Heigwer et al. 2016). According to several studies, CLCuV has the greatest influence on the seedling stage of plant, with shorter blooming time and, as a result, decreased the size and quantity of cotton bolls negatively impacting seed yield and fiber quality attributes (Ahuja et al. 2007; Qazi et al. 2007).

Genome editing has recently been utilized to target alterations in many important genes, including both endogenous or exogenous marker genes (Zhang et al. 2021b). Examples include the *GmFt2a* gene editing to postpone soybean blooming (Cai et al. 2018), the simultaneous targeted mutagenesis of three *TaEDR1* homologs to improve wheat resistance to powdery mildew (Zhang et al. 2017), similarly Li et al. (2017) used targeted mutagenesis to boost the amounts of γ-aminobutyric acid production in *Solanum lycopersicum*, and Braatz et al. (2017) used targeted mutation to decrease oilseed seed cracking by altering two *BnALC* homeologs. The genome editing technique based on CRISPR/Cas9 was also employed to provide farmed cotton resilience to biotic and abiotic stresses (Rahman et al. 2022). The establishment of genome editing systems in *G. hirsutum* mediated by CRISPR/Cas9 was described in several studies in 2017, mostly through endogenous and exogenous gene markers, such as *GFP*, *DsRed2*, and *GhMYB25*-like genes along with the *GhCLA1* gene (Chen et al. 2017; Wang et al. 2017; Janga et al. 2017; Li et al. 2017). Application of transgenic cotton, particularly cultivars resistant to insects and herbicides, has generated significant economic gains over the past 20 years (Li et al. 2024b). For example, the Chinese Bt cotton has raised annual income by 1.5 billion dollars (Zhang et al. 2024).

To offer resistance to *CLCuD* and fungal wilt caused by *Verticillium dahlia*, the CRISPR/Cas9 technology may be used to specifically interfere with *CLCuD* tolerance and wilt disease resistance and spread will be enhanced by the development of efficiently developed sgRNAs matching to the coding and non-coding genomic areas of *CLCuV* and wilt disease in Cas9 overexpressed transgenic cotton plants. This strategy will open up new possibilities for protecting cotton crops from phyto phages and fungal pathogens. Thus, the CRISPR/Cas9 genome alteration technique still needs to be used to edit intriguing and ecological genes, particularly negative regulatory genes involved in resistance and growth.

The most detrimental disease to cotton production is known as Verticillium wilt, a kind of vascular infection brought on by a fungus *Verticillium dahliae* (Gao et al. 2013; Umer et al. 2023; Zhang et al. 2022). The extensively planted upland cotton unfortunately lacks Verticillium wilt immune germplasm (Yang et al. 2015). According to Wang et al. (2016), the cotton Verticillium wilt, sometimes known as "cotton cancer" (Umer et al. 2023), is a damaging disease that causes economic damages ranging from $250 to $310 million USD each year in China. The best defense against *V. dahliae* pathogen damage to plants is to create disease-resistant cultivars. However, *G. hirsutum* naturally has only a small number of germplasm resources or resistant genes against *V. dahliae*. To specifically target negative regulators of disease resistance, such as cotton 14–3-3c/d, NINJA, and CYP82D, which, according to RNAi methods, have strong sensitivity to *V. dahlia* infestation (Wang et al. 2017; Sun et al. 2014; Gao et al. 2013), using CRISPR-Cas9 to create new defense genes or defensive mutants is essential. Zhang et al. (2018b) created a CRISPR-Cas9-mediated genome editing method in cotton and edited the target gene, *Gh14-3-3d*, to produce *in-dels* at predicted target locations. The CRISPR-Cas9 genome editing system produced many insertions and deletions of nucleotides in T_0 plants at the predicted locations within the *Gh14-3-3d* gene targets. Among the edited lines, *ce1* and *ce2* lines in T_2 have been reported to be having stronger resistance to *V. dahliae* in comparison with the wild type (Zhang et al. 2018b). This outcome can be used in raising cotton cultivars with wilt resistance against *V. dahliae*.

Cotton plants accumulate jasmonic acid (JA) and jasmonoyl-isoleucine (JA-Ile) within hours after *V. dahliae* infection, and plants with constitutively active JA

signaling are more resistant to *V. dahliae* (Hu et al. 2018). Recent studies in *Arabidopsis* have shown that the unimpeded JA signaling pathway is critical in plants resistant to *V. dahliae* (Fradin et al. 2011). Zhang et al. (2018a) observed that a *GhCPK33* gene was activated by *V. dahliae* V991. Further research showed that *GhCPK33*, which is located in the peroxisome, controls JA synthesis by phosphorylating 12-oxophytodienoate reductase 3 GhOPR3), which lowers its stability, and acts as a negative regulator of cotton defense against *V. dahliae*. Hu et al. (2018) found a phosphorylated network among *GhCPK33* and *GhOPR3*, which affects JA synthesis and the JA-dependent responses of *V. dahliae* in cotton.

By multiplex CRISPR/Cas9 technology, begomoviruses in cotton were successfully demonstrated to be controlled by targeting the viral genome of *CLCuV* (Binyameen et al. 2021; Mubarik et al. 2021). When utilizing sgRNA, the 200 bp common sequence of the DNA-A was discovered to be helpful for viral interference. The level of cotton plant resistance to *CLCuV*, further increased with the efficient strategy for concurrently targeting sgRNA expression in both coding and non-coding regions of the *CLCuV* genome (Binyameen et al. 2021; Mubarik et al. 2021). Geminivirus-based genome editing vectors, pBeYDV-Cas9-KO and pRGEB32-35S, were created by Li et al. (2022) and tested by aiming on the *GhCLA*1 gene. The multiplex CRISPR/Cas9 technology may be used to change the genomes of other members of the Geminivirdae and present a chance for more environmentally friendly agricultural production methods.

Eliminating anti-nutritional compounds

The gossypol biosynthetic pathway is responsible for the production of gossypol, a natural compound found in cotton plants (Mehari et al. 2023). Gossypol is a yellowish pigment with diverse biological activities and is primarily known for its insecticidal and antifungal properties. Understanding the characterization of the gossypol biosynthetic pathway is crucial for manipulating its production in cotton plants for various purposes. The pathway starts with the amino acid phenylalanine, it passes through a number of enzymatic reactions to produce gossypol. The key enzymes involved in this pathway, including cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), cinnamoyl-CoA reductase (CCR), phenylalanine ammonia-lyase (PAL), and cinnamyl alcohol dehydrogenase (CAD), catalyzed the conversion of phenylalanine to gossypol through several intermediate compounds.

Tian et al. (2018) have extensively studied the gossypol biosynthetic pathway to elucidate the roles of individual enzymes and regulatory factors involved in gossypol regulation. They have identified and characterized genes encoding enzymes associated with gossypol synthesis and analyzed their expression patterns across several tissues and development phases of cotton plants. Additionally, the characterization of transcription factors as well as other regulatory components that control the gene expression in the gossypol biosynthetic pathway has provided insights into the molecular mechanisms underlying gossypol production.

A study explored the use of temperature-dependent genome modification using CRISPR/LbCpf1 (LbCas12a) to generate non-transgenic cotton plants without gossypol (Li et al. 2021a). The study involved using temperature-sensitive CRISPR/LbCpf1, an alternative to the CRISPR-Cas9 mechanism, to target and modify the genes involved in gossypol biosynthesis in allotetraploid cotton (*G. hirsutum*). The temperature-sensitive feature allowed for precise control over the genome editing process. By designing specific gRNAs targeting the key genes (*GhPGF* and *GhCLA1*) in the gossypol biosynthetic pathway, targeted mutations were induced in cotton plants at the desired temperature. This led to disruptions in the expression of the gossypol biosynthesis genes and resulted in the creation of cotton plants that do not produce gossypol. The edited cotton plants demonstrated reduced levels of gossypol in seeds without compromising other important agronomic traits. The non-transgenic nature of the approach offers advantages in terms of regulatory approval and public acceptance. This research presents a significant breakthrough in developing gossypol-free cotton plants using temperature-sensitive CRISPR/LbCpf1 genome editing. The elimination of gossypol in cotton seeds enhances the potential utilization of cottonseed products in the food and feed industries (Li et al. 2021a).

Limitations and challenges of using CRISPR technology in cotton

CRISPR tools are gaining increasing popularity and are frequently used in plants. However, the use of the genome editing tools in cotton is a challenge with few limitations (Fig. 2).

Genome complexity, redundancy, and variation

Cotton has a complex genome, often being tetraploid (with four sets of chromosomes). This complexity makes it challenging to target and edit specific genes without off-target effects or unintended consequences (Manivannan et al. 2023). In a tetraploid organism like cotton, there are typically multiple copies of each gene. This redundancy can make it challenging to target a specific gene without affecting its duplicates. For example, when aiming to knock out a gene responsible

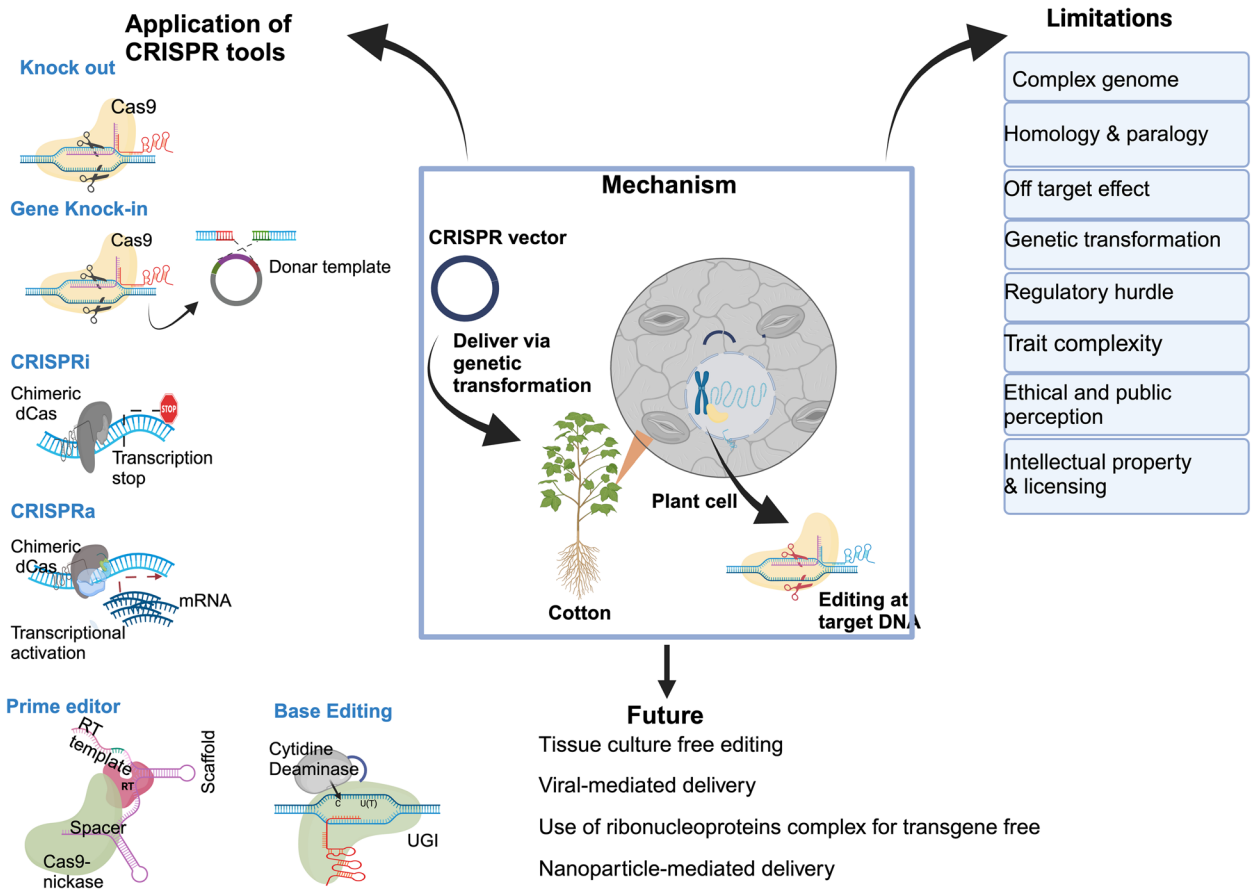

Fig. 2 Cotton genome editing and various challenges and limitations

for a specific trait, it is essential to ensure that all copies of that gene are altered, or else the trait might not be completely affected due to redundancy (Blenda et al. 2012). Tetraploid leads to a higher number of homologous and paralogous sequences; genes that are similar due to shared ancestry or duplication events. This similarity complicates the design of specific CRISPR gRNAs because the guide sequence might recognize and bind to unintended locations with similar sequences, leading to off-target effects (Liu et al. 2020).

The multiple copies of homologous genes dispersed throughout distinct homologous chromosomes also lead to a higher percentage of heterozygous mutation when the CRISPR/Cas method was utilized for editing the genome, which is why the editing efficacy of CRISPR/Cas9 in complex genomes (polyploid) like cotton is still low. Highly repetitive DNA sequences can be found in the tetraploid A and D-diploid genomes of *G. hirsutum* (Han et al. 2022; Li et al. 2015; Peng et al. 2022; Wang et al. 2018). The complexity of a tetraploid genome complicates the design and delivery of CRISPR/Cas components. A critical element is the appropriate choice of sgRNA, which has a direct impact on the effectiveness of using CRISPR/Cas9 (Ma et al. 2016).

Off-target effects

Even with advanced CRISPR techniques, there's a risk of off-target mutations. In complex genomes like that of cotton, ensuring precise editing without affecting other parts of the genome is critical but challenging (Kumar et al. 2024). Off-target cuts can lead to unintended mutations, affecting other traits or causing genetic instability.

Genetic transformation method and its efficiency

Cotton improvement has also advanced from the successful use of genome editing (Chen et al. 2021). But even with extremely low transformation efficiencies, only a small number of cotton genotypes, including YZ-1 (Yuzhao No. 1), ZM24, JIN668, and Coker312, have been transformable to date using the conventional

transformation method (*Agrobacterium*-mediated transformation) (Chen et al. 2021; Huang et al. 2021; Zhang et al. 2020; Peng et al. 2021). For the majority of current elite cotton varieties, their regeneration is recalcitrant that limits the application of transgenic and genome editing in cotton trait improvement (Ge et al. 2015; Peng et al. 2021; Zhao et al. 2022). Cotton cells can be difficult to regenerate into full plants after genetic modification (Verma et al. 2023). This hampers the overall efficiency and increases the time and resources needed to produce edited plants. However, the quick development of next generation deep sequencing (Liu et al. 2022; Lai et al. 2024), including single-cell RNA sequencing (scRNA-seq) (Pan et al. 2024), may elucidate the regulatory mechanisms controlling cotton somatic embryogenesis and plant regeneration, which will help us to develop new strategy for obtaining transgenic and CRISPR/Cas genome edited plants. Additionally, certain emerging materials, such as nanoparticles (Javaid et al. 2024; Rohela et al. 2024), may also enhance cotton plant tissue culture-based transgenics and CRISPR genome editing.

Gene expression regulation

In a tetraploid organism, gene expression is often regulated by complex mechanisms that might involve multiple gene copies. Editing one copy without considering others might not yield the desired phenotype or could have unpredictable consequences on overall gene regulation. CRISPR/Cas is a useful tool for targeting the genomes of polyploid organisms and other species with complex genomes because it can cause simultaneous mutations at many genomic locations. The sgRNAs need to be considered with care so that they can target all diverse alleles that are to be edited (Schaart et al. 2021).

Regulatory hurdles and ethical and public perception

Depending on the region, there may be stringent regulatory frameworks around genetically engineered organisms (GMOs), this might affect the deployment of CRISPR-edited cotton plants (Gupta et al. 2021). The regulations might not differentiate between traditional GMOs and gene-edited crops, complicating the approval process. Genome editing entails genetically transforming plant cells to introduce editing agents. Nevertheless, this process may affect the editing elements being randomly integrated into the genome, which might result in unfavorable genetic variations. In addition, the incorporation of foreign DNA into plant genomes poses regulatory challenges since the altered plants may be classified as genetically engineered organisms.

Despite CRISPR's technical benefits, public perception and ethical considerations around GMOs and gene editing can influence the acceptance and adoption of CRISPR-edited cotton (Munawar et al. 2023).

Trait complexity

Cotton production relies on a combination of traits, including fiber quality, resistance to pests and diseases, and yield (Zhang et al. 2021b; Wang et al. 2023). Altering one gene might not be sufficient to achieve the desired outcome without affecting other traits, requiring multiple edits and complex gene regulation (Conaty et al. 2022).

Intellectual property and licensing

Access to CRISPR technology might be restricted due to intellectual property rights, affecting researchers and institutions interested in working with cotton. Given these challenges, the successful application of CRISPR in cotton requires a multidisciplinary approach, combining advances in CRISPR technology, cautious guide RNA design, effective delivery methods, thorough analysis of potential off-target effects, and validation through extensive genomic sequencing, robust regulatory frameworks, and a clear understanding of public perception to ensure successful application in cotton and other crops (Khan et al. 2023a). Researchers often use multiple gRNAs to ensure all copies of a target gene are edited, and they rely on advanced bioinformatics tools to predict and avoid off-target effects. Additionally, regenerating edited cotton cells into full plants can be more complex due to the need for stable integration of edits across the tetraploid genome.

Conclusion and future perspectives

Genome editing using the CRISPR/Cas9 approach in cotton has enormous potential to explore gene functions and gain insights. Currently, genetic transformation of cotton plants for CRISPR/Cas delivery is achieved using laborious and time-consuming methods such as *Agrobacterium* or biolistic techniques. In the future, more efficient and rapid methods such as viral-mediated delivery, ribonucleoproteins (RNPs) complex delivery, and nanoparticle-mediated delivery will be increasingly utilized for targeted genome editing. Viral mediated delivery of CRISPR/Cas is gaining day by day popularity, where we can deliver directly the Cas endonuclease and its gRNA into leaf, stem, or flower with viruses and get a mutant seed in the next generation.

With the development of CRISPR/Cas technology, cotton can be modified more efficiently and precisely in terms of oil enhancement, fiber quality, disease resistance, herbicide tolerance, and drought resilience. Future genome editing efforts for cotton should focus on minimizing unintended genetic alterations.

Acknowledgements
The figures are made using BioRender tool (https://www.biorender.com/).

Authors' contributions
Sheri V and Mohan H: concept and draft preparation; Jogam P: table preparation. Alok A: created the figures, Rohela GK: reviewed the manuscript, and Zhang BH critically revised and corrected the article, supervision, and funding acquisition. All authors have read and approved the final manuscript.

Funding
The research performed is partially supported by the Cotton Incorporated.

Data availability
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests. Author Zhang BH is a member of the Editorial Board of Journal of Cotton Research. Author Zhang BH was not involved in the journal's review of, or decision related to this manuscript.

Received: 12 August 2024 Accepted: 20 November 2024
Published online: 03 February 2025

References

Ahmad S, Mahmood K, Hanif M, et al. Introgression of cotton leaf curl virus-resistant genes from Asiatic cotton (*Gossypium arboreum*) into upland cotton (*G. hirsutum*). Genet Mol Res. 2011;10(4):2404–14.
Ahmed AI, Khan AI, Negm MAM, et al. Enhancing cotton resilience to challenging climates through genetic modifications. J Cotton Res. 2024;7:10. https://doi.org/10.1186/s42397-024-00171-4 .
Ahuja SL, Monga D, Dhayal LS. Genetics of resistance to cotton leaf curl disease in *Gossypium hirsutum* L. under field conditions. J Hered. 2007;98(1):79–83. https://doi.org/10.1093/jhered/esl049 .
Aini N, Jibril AN, Liu S, et al. Advances and prospects of genetic mapping of Verticillium wilt resistance in cotton. J Cotton Res. 2022;5:5. https://doi.org/10.1186/s42397-021-00109-0 .
Ali I, Amin I, Briddon RW, et al. Artificial microRNA-mediated resistance against the monopartite begomovirus cotton leaf curl Burewala virus. Virol J. 2013;10:231.
Aslam S, Khan SH, Ahmad A, et al. Founder transformants of cotton (*Gossypium hirsutum* L.) obtained through the introduction of DS-Red, Rec, Rep and CRISPR/Cas9 expressing constructs for developing base lines of recombinase mediated gene stacking. PLoS One. 2022;17(2):e0263219. https://doi.org/10.1371/journal.pone.0263219 .
Bassett AR, Tibbit C, Ponting CP, et al. Highly efficient targeted mutagenesis of *Drosophila* with the CRISPR/Cas9 system. Cell Rep. 2013;4(1):220–8. https://doi.org/10.1016/j.celrep.2013.06.020 .
Binyameen B, Khan Z, Khan SH, et al. Using multiplexed CRISPR/Cas9 for suppression of cotton leaf curl virus. Int J Mech Sci. 2021;22(22):12543.
Blenda A, Fang DD, Rami JF, et al. A high-density consensus genetic map of tetraploid cotton that integrates multiple component maps through molecular marker redundancy check. PLoS One. 2012;7(9):e45739. https://doi.org/10.1371/journal.pone.0045739 .
Braatz J, Harloff HJ, Mascher M, et al. CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (*Brassica napus*). Plant Physiol. 2017;174(2):935–42. https://doi.org/10.1104/pp.17.00426 .
Cai Y, Chen L, Liu X, et al. CRISPR/Cas9-mediated targeted mutagenesis of *GmFT2a* delays flowering time in soya bean. Plant Biotechnol J. 2018;16(1):176–85. https://doi.org/10.1111/pbi.12758 .
Chen X, Lu X, Shu N, et al. Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. Sci Rep. 2017;7(1):1–7. https://doi.org/10.1038/srep44304 .
Chen Y, Fu M, Li H, et al. High-oleic acid content, nontransgenic allotetraploid cotton (*Gossypium hirsutum* L.) generated by knockout of *GhFAD2* genes with CRISPR/Cas9 system. Plant Biotechnol J. 2021;19(3):424–6. https://doi.org/10.1111/pbi.13507 .
Conaty WC, Broughton KJ, Egan LM, et al. Cotton breeding in Australia: meeting the challenges of the 21st century. Front Plant Sci. 2022;13:904131.
Dochia M, Sirghie C, Kozłowski RM, et al. Cotton fibres. In: Kozłowski RM, Mackiewicz-Talarczyk M, editors. Handbook of natural fibres. Cambridge, England: Woodhead Publishing; 2012. p. 11–23.
Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096. https://doi.org/10.1126/science.1258096 .
Fang S, Shang X, He Q, et al. A cell wall–localized β-1,3-glucanase promotes fiber cell elongation and secondary cell wall deposition. Plant Physiol. 2024;194(1):106–23. https://doi.org/10.1093/plphys/kiad407.
Fradin EF, Abd-El-Haliem A, Masini L, et al. Interfamily transfer of tomato *Ve1* mediates *Verticillium* resistance in *Arabidopsis*. Plant Physiol. 2011;156(4):2255–65. https://doi.org/10.1104/pp.111.180067 .
Gao W, Long L, Tian X, et al. Genome editing in cotton with the CRISPR/Cas9 system. Front Plant Sci. 2017;8:290219.
Gao W, Long L, Zhu LF, et al. Proteomic and virus-induced gene silencing (VIGS) analyses reveal that gossypol, brassinosteroids, and jasmonic acid contribute to the resistance of cotton to *Verticillium dahliae*. Mol Cell Proteomics. 2013;12(12):3690–703. https://doi.org/10.1074/mcp.M113.031013 .
Ge X, Xu J, Yang Z, et al. Efficient genotype-independent cotton genetic transformation and genome editing. J Integr Plant Biol. 2023;65(4):907–17. https://doi.org/10.1111/jipb.13427 .
Ge X, Zhang C, Wang Q, et al. iTRAQ protein profile differential analysis between somatic globular and cotyledonary embryos reveals stress, hormone, and respiration involved in increasing plantlet regeneration of *Gossypium hirsutum* L. J Proteome Res. 2015;14(1):268–78. https://doi.org/10.1021/pr500688g .
Gong J, Sun S, Zhu QH, et al. *Gh4CL20/20A* involved in flavonoid biosynthesis is essential for male fertility in cotton (*Gossypium hirsutum* L.). Plant Physiol Biochem. 2024;208:108484. https://doi.org/10.1016/j.plaphy.2024.108484 .
Gupta S, Kumar A, Patel R, et al. Genetically modified crop regulations: scope and opportunity using the CRISPR-Cas9 genome editing approach. Mole Biol Rep. 2021;48(5):4851–63. https://doi.org/10.1007/s11033-021-06477-9 .
Gupta A, Kumar M, Zhang B, et al. Improvement of qualitative and quantitative traits in cotton under normal and stressed environments using genomics and biotechnological tools: a review. Plant Sci. 2024;340:111937. https://doi.org/10.1016/j.plantsci.2023.111937.
Han J, Lopez-Arredondo D, Yu G, et al. Genome-wide chromatin accessibility analysis unveils open chromatin convergent evolution during polyploidization in cotton. Proc Natl Acad Sci USA. 2022;119(44):e2209743119.
Heigwer F, Zhan T, Breinig M, et al. CRISPR library designer (CLD): Software for multispecies design of single guide RNA libraries. Geno Biol. 2016;17:55. https://doi.org/10.1186/s13059-016-0915-2 .
Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262–78. https://doi.org/10.1016/j.cell.2014.05.010 .
Huang G, Huang JQ, Chen XY, et al. Recent advances and future perspectives in cotton research. Annu Rev Plant Biol. 2021;72(1):437–62. https://doi.org/10.1146/annurev-arplant-080720-113241 .
Hu Q, Zhu L, Zhang X, et al. *GhCPK33* negatively regulates defense against *Verticillium dahliae* by phosphorylating *GhOPR3*. Plant Physiol. 2018;178(2):876–89. https://doi.org/10.1104/pp.18.00737 .
Hussain S, Ahmad A, Wajid A, et al. Irrigation scheduling for cotton cultivation. In: Ahmad S, Hasanuzzaman M, editors. Cotton production and uses: agronomy, crop protection, and postharvest technologies. Singapore: Springer; 2020. p. 59–80.

Iqbal Z, Sattar MN, Shafiq M. CRISPR/Cas9: a tool to circumscribe cotton leaf curl disease. Front Plant Sci. 2016;7:190600.
Janga MR, Campbell LAM, Rathore KS. CRISPR/Cas9-mediated targeted mutagenesis in upland cotton (*Gossypium hirsutum* L.). Plant Mol Biol. 2017;94(4–5):349–60. https://doi.org/10.1007/s11103-017-0599-3 .
Janga MR, Pandeya D, Campbell LAM, et al. Genes regulating gland development in the cotton plant. Plant Biotechnol J. 2019;17(6):1142–53. https://doi.org/10.1111/pbi.13044 .
Javaid A, Hameed S, Li L, et al. Can nanotechnology and genomics innovations trigger agricultural revolution and sustainable development? Funct Integr Genomics. 2024;24:216. https://doi.org/10.1007/s10142-024-01485-x .
Jogam P, Sandhya D, Alok A, et al. A review on CRISPR/Cas-based epigenetic regulation in plants. Int J Biol Macromol. 2022;219:1261–71. https://doi.org/10.1016/j.ijbiomac.2022.08.182 .
Khan Z, Khan SH, Ahmed A, et al. Genome editing in cotton: challenges and opportunities. J Cotton Res. 2023a;6:3. https://doi.org/10.1186/s42397-023-00140-3 .
Khan AH, Ma Y, Wu Y, et al. High-temperature stress suppresses allene oxide cyclase 2 and causes male sterility in cotton by disrupting jasmonic acid signaling. Crop J. 2023b;11(1):33–45.
Kiobia DO, Mwitta CJ, Fue KG, et al. A review of successes and impeding challenges of IoT-based insect pest detection systems for estimating agro-ecosystem health and productivity of cotton. Sensors. 2023;23(8):4127. https://doi.org/10.3390/s23084127 .
Kumar M, Zhang B, Potkule J, et al. Cottonseed oil: extraction, characterization, health benefits, safety profile, and application. Food Anal Methods. 2023;16(2):266–280. https://doi.org/10.1007/s12161-022-02410-3.
Kumar R, Das J, Puttaswamy RK, et al. Targeted genome editing for cotton improvement: prospects and challenges. Nucleus (India). 2024;67(1):181–203. https://doi.org/10.1007/s13237-024-00479-1 .
Lai Y, Ma J, Zhang X, et al. High-quality chromosome-level genome assembly and multi-omics analysis of rosemary (*Salvia rosmarinus*) reveals new insights into the environmental and genome adaptation. Plant Biotechnol J. 2024;22(7):1833–47. https://doi.org/10.1111/pbi.14305 .
Li T, Long L, Tang Y, et al. Knock-out of *GhPDCT* with the CRISPR/Cas9 system increases the oleic acid content in cottonseed oil. J Integr Agr. 2024a;23(10):3468–71.
Lee JJ, Woodward AW, Chen ZJ. Gene expression changes and early events in cotton fibre development. Ann Bot. 2007;100(7):1391–401. https://doi.org/10.1093/aob/mcm232 .
Lei J, Li Y, Dai P, et al. Efficient virus-mediated genome editing in cotton using the CRISPR/Cas9 system. Front Plant Sci. 2022;13:1032799.
Li B, Fu C, Zhou J, et al. Highly efficient genome editing using geminivirus-based CRISPR/Cas9 system in cotton plant. Cells. 2022;11(18):2902. https://doi.org/10.3390/cells11182902 .
Li B, Rui H, Li Y, et al. Robust CRISPR/Cpf1 (Cas12a)-mediated genome editing in allotetraploid cotton (*Gossypium hirsutum*). Plant Biotechnol J. 2019a;17(10):1862–4. https://doi.org/10.1111/pbi.13147 .
Li C, Brant E, Budak H, et al. CRISPR/Cas: a Nobel Prize award-winning precise genome editing technology for gene therapy and crop improvement. J Zhejiang Univ Sci B. 2021b;22(4):253–84. https://doi.org/10.1631/jzus.B2100009 .
Li C, Chu W, Gill RA, et al. Computational tools and resources for CRISPR/Cas genome editing. Genomics Proteomics Bioinformatics. 2023;21(1):108–26. https://doi.org/10.1016/j.gpb.2022.02.006 .
Li C, Zhang B. Genome editing in cotton using CRISPR/Cas9 system. Methods Mol Biol. 2019d;1902:95–104. https://doi.org/10.1007/978-1-4939-8952-2_8 .
Li F, Fan G, Lu C, et al. Genome sequence of cultivated upland cotton (*Gossypium hirsutum* TM-1) provides insights into genome evolution. Nat Biotechnol. 2015;33(5):524–30. https://doi.org/10.1038/nbt.3208 .
Li C, Unver T, Zhang B. A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in cotton (*Gossypium hirsutum* L.). Sci Rep. 2017;71:43902. https://doi.org/10.1038/srep43902 .
Li J, Manghwar H, Sun L, et al. Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants. Plant Biotechnol J. 2019b;17(5):858–68. https://doi.org/10.1111/pbi.13020 .
Li J, Wang M, Li Y, et al. Multi-omics analyses reveal epigenomics basis for cotton somatic embryogenesis through successive regeneration acclimation process. Plant Biotechnol J. 2019c;17(2):435–50. https://doi.org/10.1111/pbi.12988 .
Li B, Liang S, Alariqi M, et al. The application of temperature sensitivity CRISPR/LbCpf1 (LbCas12a) mediated genome editing in allotetraploid cotton (*G. hirsutum*) and creation of nontransgenic, gossypol-free cotton. Plant Biotechnol J. 2021a;19(2):221–3. https://doi.org/10.1111/pbi.13470 .
Li LJ, Zhang DQ, Zhang ZY, et al. CRISPR/Cas: a powerful tool for designing and improving oil crops. Trends Biotechnol. 2024a. https://doi.org/10.1016/j.tibtech.2024.09.007.
Li LJ, Zhang ZY, Zhang BH. iJAZ: the next breakthrough for engineering pest-resistance in plants? Trends Plant Sci. 2024b;29(12):1282–4. https://doi.org/10.1016/j.tplants.2024.07.013 .
Lin JL, Fang X, Li JX, et al. Dirigent gene editing of gossypol enantiomers for toxicity-depleted cotton seeds. Nat Plants. 2023;9(4):605–15. https://doi.org/10.1038/s41477-023-01376-2 .
Liu G, Zhang Y, Zhang T. Computational approaches for effective CRISPR guide RNA design and evaluation. Comput Struct Biotechnol J. 2020;1(18):35–44.
Liu W, Zhang BH. The landscape of genome sequencing and assembling in plants. Funct Integr Genomics. 2022;22:1147–52. https://doi.org/10.1007/s10142-022-00916-x .
Long L, Feng YM, Shang SZ, et al. *In vivo* maternal haploid induction system in cotton. Plant Physiol. 2024;194(3):1286–9. https://doi.org/10.1093/plphys/kiad620 .
Long L, Guo DD, Gao W, et al. Optimization of CRISPR/Cas9 genome editing in cotton by improved sgRNA expression. Plant Methods. 2018;14:85. https://doi.org/10.1186/s13007-018-0353-0 .
Ma D, Hu Y, Yang C, et al. Genetic basis for glandular trichome formation in cotton. Nat Commun. 2016;7(1):10456. https://doi.org/10.1038/ncomms10456 .
Manivannan A, Cheeran AT. Deciphering the complex cotton genome for improving fiber traits and abiotic stress resilience in sustainable agriculture. Mol Biol Rep. 2023;50(8):6937–53. https://doi.org/10.1007/s11033-023-08565-4 .
Mehari TG, Fang H, Feng W, et al. Genome-wide identification and expression analysis of terpene synthases in *Gossypium* species in response to gossypol biosynthesis. Funct Integr Genomics. 2023;23:197. https://doi.org/10.1007/s10142-023-01125-w.
Morris H, Murray R. Medical textiles. Text Prog. 2020;52(1–2):1–127. https://doi.org/10.1080/00405167.2020.1824468 .
Mubarik MS, Wang X, Khan SH, et al. Engineering broad-spectrum resistance to cotton leaf curl disease by CRISPR-Cas9 based multiplex editing in plants. GM Crops Food. 2021;12(2):647–58. https://doi.org/10.1080/21645698.2021.1938488 .
Munawar N, Ahsan K, Ahmad A. CRISPR-edited plants' social, ethical, policy, and governance issues. In: Abd-ElsalamKA, Ahmad A, editors. Global regulatory outlook for CRISPRized plants. Amsterdam, Netherlands: Elsevier; 2023. p. 367–96.
Oliveira MAC, Duarte JB, Morello CL, et al. Mixed inheritance in the genetic control of ramulosis (*Colletotrichum gossypii* var. cephalosporioides) resistance in cotton. Genet Mole Res. 2016;15(3). https://doi.org/10.4238/gmr.15038667 .
Pan Y, Meng F, Wang X. Sequencing multiple cotton genomes reveals complex structures and lays foundation for breeding. Front Plant Sci. 2020;11:560096.
Pan X, Peng R, Zhang BH. Single-cell RNA sequencing opens a new era for cotton genomic research and gene functional analysis. J Cotton Res. 2024;7:17. https://doi.org/10.1186/s42397-024-00181-2 .
Peng R, Jones DC, Liu F, et al. From sequencing to genome editing for cotton improvement. Trends Biotechnol. 2021;39(3):221–4. https://doi.org/10.1016/j.tibtech.2020.09.001 .
Peng R, Xu Y, Tian S, et al. Evolutionary divergence of duplicated genomes in newly described allotetraploid cottons. Proc Natl Acad Sci USA. 2022;119(39):e2208496119. https://doi.org/10.1073/pnas.2208496119 .
Prabhu KH, Bhute AS. Plant based natural dyes and mordnats: a review. J Nat Prod Plant Resour. 2012;6:649–64.
Prakash S, Kumar M, Radha, et al. The resilient cotton plant: uncovering the effects of stresses on secondary metabolomics and its underlying molecular mechanisms. Funct Integr Genomics. 2023;23(2):183. https://doi.org/10.1007/s10142-023-01118-9 .

Qazi J, Amin I, Mansoor S, et al. Contribution of the satellite encoded gene βC1 to cotton leaf curl disease symptoms. Virus Res. 2007;128(1–2):135–9. https://doi.org/10.1016/j.virusres.2007.04.002 .
Qin L, Li J, Wang Q, et al. High-efficient and precise base editing of C•G to T•A in the allotetraploid cotton (*Gossypium hirsutum*) genome using a modified CRISPR/Cas9 system. Plant Biotechnol J. 2020;18(1):45–56. https://doi.org/10.1111/pbi.13168 .
Rahman M-u. Zulfiqar S, Raza MA, et al. Engineering abiotic stress tolerance in crop plants through CRISPR genome editing. Cells, 2022;11(22):3590. https://doi.org/10.3390/cells11223590.
Rohela GK, Saini P, Aziz D, et al. Nanoparticles as elicitors and stimulators for plant tissue culture, transgenics, and genome editing: a comprehensive review. Ind Crops Prod. 2024;222:120097. https://doi.org/10.1016/j.indcrop.2024.120097 .
Saeed F, Chaudhry UK, Raza A, et al. Developing future heat-resilient vegetable crops. Funct Integr Genomics. 2023;23:47. https://doi.org/10.1007/s10142-023-00967-8 .
Samada LH, Tambunan USF. Biopesticides as promising alternatives to chemical pesticides: a review of their current and future status. Online J Biol Sci. 2020;20(2):66–76. https://doi.org/10.3844/ojbsci.2020.66.76 .
Sandhya D, Jogam P, Allini VR, et al. The present and potential future methods for delivering CRISPR/Cas9 components in plants. J Genet Eng Biotechnol. 2020;18:25. https://doi.org/10.1186/s43141-020-00036-8 .
Schaart JG, van de Wiel CCM, Smulders MJM. Genome editing of polyploid crops: prospects, achievements and bottlenecks. Transgenic Res. 2021;30(4):337–51. https://doi.org/10.1007/s11248-021-00251-0 .
Shang X, Cheng C, Ding J, et al. Identification of candidate genes from the SADgene family in cotton for determination of cottonseed oil composition. Mol Genet Genomics. 2017;292(1):173–86. https://doi.org/10.1007/s00438-016-1265-1 .
Sheri V, Kumar M, Jaconis S, et al. Antioxidant defense in cotton under environmental stresses: unraveling the crucial role of a universal defense regulator for enhanced cotton sustainability. Plant Physiol Biochem. 2023;204:10141. https://doi.org/10.1016/j.plaphy.2023.108141 .
Shuli F, Jarwar AH, Wang X, et al. Overview of the cotton in Pakistan and its future prospects. Pak J Agric Res. 2018;31(4):396–407. https://doi.org/10.17582/journal.pjar/2018/31.4.396.407 .
Singh RP, Prasad PVV, Sunita K, et al. Influence of high temperature and breeding for heat tolerance in cotton: a review. Adv Agro. 2007;93:313–85. https://doi.org/10.1016/S0065-2113(06)93006-5 .
Sun L, Zhu L, Xu L, et al. Cotton cytochrome P450 CYP82D regulates systemic cell death by modulating the octadecanoid pathway. Nat Commun. 2014;5:5372. https://doi.org/10.1038/ncomms6372 .
Sun L, Alariqi M, Zhu Y, et al. Red fluorescent protein (DsRed2), an ideal reporter for cotton genetic transformation and molecular breeding. Crop J. 2018;6(4):366–76.
Tian X, Ruan JX, Huang JQ, et al. Characterization of gossypol biosynthetic pathway. Proc Natl Acad Sci U S A. 2018;115(23):E5410–8. https://doi.org/10.1073/pnas.1805085115 .
Todor MP, Kiss I, Cioata VG. Development of fabric-reinforced polymer matrix composites using bio-based components from post-consumer textile waste. Mater Today: Proc. 2021;45:4150–6.
Umer MJ, Zheng J, Yang M, et al. Insights to *Gossypium* defense response against *Verticillium dahliae*: the cotton cancer. Funct Integr Genomics. 2023;23(2):142. https://doi.org/10.1007/s10142-023-01065-5.
Verma K, Sharma P, Tripathi K, et al. Recent advances in genetic improvement of cotton. In: Tiwari S, Koul B, editors. Genetic engineering of crop plants for food and health security. Singapore: Springer; 2023. p. 69–99. https://doi.org/10.1007/978-981-99-5034-8_4 .
Wang Y, Liang C, Wu S, et al. Significant improvement of cotton Verticillium wiltresistance by manipulating the expression of Gastrodia antifungal proteins. Mol Plant. 2016;9(10):1436–9. https://doi.org/10.1016/j.molp.2016.06.013 .
Wang Y, Meng Z, Liang C, et al. Increased lateral root formation by CRISPR/Cas9-mediated editing of arginase genes in cotton. Sci China Life Sci. 2017;60(5):524–7.https://doi.org/10.1007/s11427-017-9031-y .
Wang P, Zhang J, Sun L, et al. High efficient multisites genome editing in allotetraploid cotton (*Gossypium hirsutum*) using CRISPR/Cas9 system. Plant Biotechnol J. 2018;16(1):137–50. https://doi.org/10.1111/pbi.12755 .
Wang Q, Alariqi M, Wang F, et al. The application of a heat-inducible CRISPR/Cas12b (C2c1) genome editing system in tetraploid cotton (*G. hirsutum*) plants. Plant Biotechnol J. 2020;18(12):2436–43. https://doi.org/10.1111/pbi.13417 .
Wang Y, Guo X, Xu Y, et al. Genome-wide association study for boll weight in *Gossypium hirsutum* races. Funct Integr Genomics. 2023;23:331. https://doi.org/10.1007/s10142-023-01261-3.
Wang G, Wang F, Xu Z, et al. Precise fine-turning of *GhTFL1* by base editing tools defines ideal cotton plant architecture. Genome Biol. 2024;25:59. https://doi.org/10.1186/s13059-024-03189-8 .
Wei X, Geng M, Yuan J, et al. GhRCD1 promotes cotton tolerance to cadmium by regulating the GhbHLH12–GhMYB44–GhHMA1 transcriptional cascade. Plant Biotechnol J. 2024;22(7):1777–96. https://doi.org/10.1111/pbi.14301 .
Wilson J. Fibres, yarns and fabrics: fundamental principles for the textile designer. In: Clarke S, editor. Textile Design. London, England: Laurence King; 2011. p. 3–30.
Wu M, Pei W, Wedegaertner T, et al. Genetics, breeding and genetic engineering to improve cottonseed oil and protein: a review. Front Plant Sci. 2022;13:864850.
Wu C, Xiao S, Zhang X, et al. GhHDZ76, a cotton HD-Zip transcription factor, involved in regulating the initiation and early elongation of cotton fiber development in *G. hirsutum*. Plant Sci. 2024;345:112132. https://doi.org/10.1016/j.plantsci.2024.112132 .
Xu Y, Wei Y, Zhou Z, et al. Widespread incomplete lineage sorting and introgression shaped adaptive radiation in the *Gossypium* genus. Plant Commun. 2024;5(2):100728. https://doi.org/10.1016/j.xplc.2023.100728 .
Yang CL, Liang S, Wang HY, et al. Cotton major latex protein 28 functions as a positive regulator of the ethylene responsive factor 6 in defense against *Verticillium dahliae*. Mol Plant. 2015;8(3):399–411.https://doi.org/10.1016/j.molp.2014.11.023.
Yang Y, Zhou X, Zhu X, et al. GhMYB52 like: a key factor that enhances lint yield by negatively regulating the lignin biosynthesis pathway in fibers of upland cotton (*Gossypium hirsutum* L.). Int J Mol Sci. 2024;25(9):4921. https://doi.org/10.3390/ijms25094921 .
Yu L, Li Z, Ding X, et al. Developing an efficient CRISPR–dCas9–TV-derived transcriptional activation system to create three novel cotton germplasm materials. Plant Commun. 2023;4(4):100600. https://doi.org/10.1016/j.xplc.2023.100600.
Yuan D, Tang Z, Wang M, et al. The genome sequence of sea-island cotton (*Gossypium barbadense*) provides insights into the allopolyploidization and development of superior spinnable fibres. Sci Rep. 2015;5:17662. https://doi.org/10.1038/srep17662 .
Zang Y, Xu C, Yu L, et al. GHCU, a molecular chaperone, regulates leaf curling by modulating the distribution of KNGH1 in cotton. Adv Sci. 2024;11(26):2402816. https://doi.org/10.1002/advs.202402816 .
Zhang J, Wedegaertner T. Genetics and breeding for glandless upland cotton with improved yield potential and disease resistance: a review. Front Plant Sci. 2021b;12:753426.
Zhang T, Hu Y, Jiang W, et al. Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. acc. TM-1) provides a resource for fiber improvement. Nat Biotechnol. 2015;33(5):531–7. https://doi.org/10.1038/nbt.3207 .
Zhang Y, Bai Y, Wu G, et al. Simultaneous modification of three homoeologs of *TaEDR1* by genome editing enhances powdery mildew resistance in wheat. Plant J. 2017;91(4):714–24. https://doi.org/10.1111/tpj.13599 .
Zhang J, Wu P, Li N, et al. A male-sterile mutant with necrosis-like dark spots on anthers was generated in cotton. Front Plant Sci. 2023;13:1102196.
Zhang L, Wang M, Li N, et al. Long noncoding RNAs involve in resistance to *Verticillium dahliae*, a fungal disease in cotton. Plant Biotechnol J. 2018a;16(6):1172–85.
Zhang D, Chen C, Wang H, et al. Cotton fiber development requires the pentatricopeptide repeat protein GhIm for splicing of mitochondrial *nad7* mRNA. Genetics. 2021a;217(1):iyaa017. https://doi.org/10.1093/genetics/iyaa017 .
Zhang D, Zhang Z, Unver T, et al. CRISPR/Cas: A powerful tool for gene function study and crop improvement. J Adv Res. 2021c;29:207–21. https://doi.org/10.1016/j.jare.2020.10.003.
Zhang YJ, Dong HZ. Resolved concerns after 28 years of *Bt* cotton in China. J Cotton Res. 2024;7:29. https://doi.org/10.1186/s42397-024-00194-x .
Zhang Y, Zhou J, Zhao L, et al. A review of the pathogenicity mechanism of *Verticillium dahliae* in cotton. J Cotton Res. 2022;5:3.https://doi.org/10.1186/s42397-021-00111-6 .

Zhang Z, Ge X, Luo X, et al. Simultaneous editing of two copies of *GH14–3–3D* confers enhanced transgene-clean plant defense against *Verticillium dahliae* in allotetraploid upland cotton. Front Plant Sci. 2018b;9:356214.
Zhao G, Li W, Xu M, et al. *GhWER* controls fiber initiation and early elongation by regulating ethylene signaling pathway in cotton (*Gossypium hirsutum*). Mol breed. 2024;44(6):38. https://doi.org/10.1007/s11032-024-01477-6 .
Zhao H, Ge X, Li F. Revitalize China's cotton industry. Nature. 2022;604(7907):625. https://doi.org/10.1038/d41586-022-01106-2 .
Zhu S, Yu X, Li Y, et al. Highly efficient targeted gene editing in upland cotton using the CRISPR/Cas9 system. Int J Mol Sci. 2018;19(10):3000. https://doi.org/10.3390/ijms19103000 .
Zhu S, Li Y, Zhang X, et al. *GhAlaRP*, a cotton alanine rich protein gene, involves in fiber elongation process. Crop J. 2021;9(2):313–24. https://doi.org/10.1016/j.cj.2020.08.007 .
Zhu QH, Jin S, Yuan Y, et al. CRISPR/Cas9-mediated saturated mutagenesis of the cotton *MIR482* family for dissecting the functionality of individual members in disease response. Plant Direct. 2022;6(6):e410. https://doi.org/10.1002/pld3.410 .