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RESEARCHARTICLE

Trends in Biotechnology and Plant Sciences

Advanced Breeding Techniques in Cotton

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Abstract
Cotton is one of the most economical crops which provides the world with good quality fiber, oil and animal feed. For the production of high quality and yield of fiber new varieties of cotton are produced. They also possess resistance against diseases and pests. There are many traditional and modern techniques that are used for the production of new varieties containing genes for better yield and resistance. In past many conventional methods were used for the breeding of cotton, such as back crossing, hybridization, polyploidization, mutational breeding. But now, there are many modern techniques available for the breeding of plants, removal, or addition of genes of interest in the genome of a selected plant species. Many markers are used to identify the location of a specific gene, which can be further isolated and inserted into some other genome. Genome editing, zinger finger nucleases, TALENS, and CRISPR Cas. In the future there will be new technologies for the improvement of crops and economical plants

KEYWORDS
Polyploidy, Fiber quality, Conventional breeding, Genetic improvement.

1 |INTRODUCTION

More than 50 countries grow cotton for the high quality of fiber from it and variety of other uses such as oil and cloth industry and is grown at commercial level for cash, mainly in Pakistan, India, China, USA, Uzbekistan etc (Khadi et al., 2010). An ideal system to study the gene pool, evolution of gene, and elongation of cells is available in cotton (Huang et al., 2021). Cotton belongs to the genus *Gossypium* which belongs to the family Malvaceae. It has the capability to produces most important textile fiber, fulfilling ~35% of the world's total annual fiber demands (Huang & Zhu, 2019). Cotton is a tropically grown as an annual crop for fiber (lint), oil and meal for animal feed. Its growth habit means a production season is of longer duration up to 180 days (Constable & Bange, 2015). A few members of the Gossypium (cotton) genus are cultivated for the production of elongated single-celled fibers valued worldwide at about $20 billion annually at the farm gate, and which sustain one of the world's largest industries (textiles) with an annual worldwide economic impact of about $500 billion. In a number of ways, cotton production and the textile industry are closely tied to petrochemical usage. At a practical level, cotton genomics offers both bio-based carbon sequestration alternatives to petrochemical use, and improved sustainability of crop production (Paterson, 2009).

The cotton genus (*Gossypium*) contains more than 50 species distributed in arid to semiarid regions of the tropic and subtropics. Following the genus origin approximately 10–15 million years ago, a rapid global radiation leads to eight major genome groups (A through G and K) of diploids (n = 13) (Hu et al., 2022) and one allopolyploid group (AD). Hybridization and polyploidization of two parental diploids, an A genome-like species with a D genome-like species, has resulted in seven allotetraploid species. Two allotetraploid species, *Gossypium hirsutum* and *Gossypium barbadense*, evolved independently; these two account for over 90% of annual commercial fiber production (Hu & Wendel, 2019). *Gossypium hirsutum* is grown primarily because of its relatively high productivity and wide adaptability. Approximately 91% or more of the world production is planted to cultivars of *G. hirsutum* types, many of which were derived from American Upland cultivars. Hybridization among types of *Gossypium barbadense* and with *G. hirsutum* broadened the germplasm base sufficiently for significant improvement in plant type, production and maturity, while maintaining fiber quality. Developmental breeding has been a very productive endeavor in cotton, and has contributed much to the evolution of cultivars that possess new or improved

characteristics of importance to the cotton industry (Niles & Feaster, 2016).

Biotechnology and plant breeding go hand-in-hand to develop new varieties of crops, which not only confer resistance or protection against pests, but also have nutritional benefits. Genetically modified (GM) crops are genetically engineered to provide desirous trait, specifically designed in agricultural beneficial scenario. GM crops are now an important part of agriculture productivity with low input and high output at field level. Highly susceptible to most of the insects, GM cotton, resistant to pest is the most globally cultivated crop. Other GM crops like, rice, papaya, sugar beet, brinjal and maize have been commercially cultivated in one or the other countries. However, contrary to several other countries allowing cultivation of GM crops, Bt cotton remains the only GM crop grown in India and Pakistan (Deshmukh, 2021).

Development of cottons that mature early (short-season cottons) and possess enhanced host plant resistance received much attention from about 1970 until the mid-1990s, but emphases on these traits have subsided with the advent of transgenic Bt cottons and boll weevil eradication. Improvements in host plant resistance, yield selection, and fiber quality are now frequently being facilitated by DNA markers, test site selection via genotype × environment (G × E), and advances in fiber testing equipment, respectively. Basic methodology of conventional cotton breeding still involves crossing diverse parents, making early-generation selections, and then evaluating subsequently developed strains, but now adventitious presence of transgenes must be carefully avoided. Increased lint yield is still the major selection criterion used in conventional breeding programs. Selection and evaluation of yield components, growth habit, maturity, and host plant resistance traits are often employed to enhance yield and/or yield stability. New fiber testing methods have permitted breeders to place more attention on improving fiber quality and to make progress in overcoming the historical negative relationships between yield and fiber quality. Continued genetic improvements in conventional cottons will occur as conventional cotton breeders continue to develop and employ enhanced methods and approaches (Bourland & Myers, 2015).

Conventional Breeding

Plant breeding can be defined as a process wherein, specific heritable changes are induced in plants through human efforts (Orton, 2020). It is an ongoing attempt in developing plants of superior phenotypes which produce more yield, resistance to diseases and abiotic stressors, synchronous maturity etc(Bhargava & Srivastava, 2019). Breeding of yesterday or conventional plant breeding refers to techniques other than modern biotechnology, in particular cross-breeding, back-crossing, etc (Negm, 2020).

The essential value of backcrossing is that it, provides a means of limiting the heterogeneity which we would result from 'straight' crosses between two types, making it possible to produce a hybrid similar to whichever of the two varieties has the more valuable genetic constitution, yet containing desirable characters transferred from the other parent. Backcrossing obviates the necessity for rigid selection generation after generation by progressively and automatically, rendering the hybrid more and more homogeneous. In many ways it is, to the plant breeder, the equivalent of line breeding to the stock breeder, with the added advantage that many plants are not harmed even by the closest inbreeding (Knight, 1945).

The objective of hybridization is to combine desirable traits from two or more parents into a single cultivar. This follows after the existing natural variation is exhausted (Yali & Mitiku, 2022a).The first breeding successes were achieved by utilizing spontaneous (naturally occurring) mutations. The most well-known example is the use of semi-dwarf wheat and rice mutants during the 'Green Revolution.' Induced mutagenesis is becoming increasingly popular in plant molecular biology as a method for identifying and isolating genes, as well as studying their structure and function. Molecular mutation breeding is ushering in a new era of crop enhancement mutation breeding (Yali & Mitiku, 2022b). At present, cotton improvement efforts rely largely on traditional breeding approaches, which have led to improved fiber yield and quality (Guzman et al., 2021). Achieving additional genetic gains through traditional breeding methods may prove challenging but early molecular breeding strategies have shown that genomic selection can improve efficiency (Islam et al., 2020). Currently, with techniques like genome sequencing and editing, the plant breeding industry has further been improved and has seen huge profits (Orton, 2020).

Modern Breeding Techniques

For last 20 years modern technologies are being amalgamated with conventional breeding practices. With time the breeding objectives of plants are moving beyond the limits to improve crop yield only. More unique traits like weed resistant, improved nutrition and responsiveness to soil and microbial community are studied robustly (Fu, 2015). There was a need for Modern breeding techniques due to the extensive time taken for Conventional breeding techniques. Sexual incompatibility and the sexual barriers in the form of pre and post fertilization were also major concerns (Bhargava & Srivastava, 2019). In order to achieve higher genetic gains, genomic selection, enviromics and

High Throughput Phenotyping (HTP) were utilized. It was also said that “Modern plant- breeding Triangle” consists of genomics, phenomics and enviromics (Crossa et al., 2017).

Marker Assisted Selection

DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS). The large number of quantitative trait loci (QTLs) mapping studies for diverse crops species have provided an abundance of DNA marker–trait associations (Collard & Mackill, 2008).

There are five main considerations for the use of DNA markers in MAS: reliability; quantity and quality of DNA required; technical procedure for marker assay; level of polymorphism; and cost (Mackill et al., 1999). Marker should be tightly linked to the loci. Large and high quality of DNA is required. Simple and less time-consuming techniques are used. Marker should be highly polymorphic and cost effective (Anand et al., 2023). There are many types of marker such as molecular such as DNA marker, morphological, biochemical and cytological markers.(Bhargava & Srivastava, 2019). The advantages of MAS may have a deep impact on crop plant breeding in the future and may change the crop plant breeding paradigm (Koebner & Summers, 2003).

Molecular markers based on PCR techniques do not require a probe hybridization step (Yang et al., 2015). Short sequences of nucleotides that are attached with the DNA to synthesize the full length of dsDNA are called primers. Most of the primers are used for the selection of specific regions of DNA to be amplified by polymerase chain reaction and sequence analysis techniques (Freeland, 2017). DNA sequences change their position in the genome and may insert into the coding regions of the genome. Such mobile DNA sequences are called transposable elements (TE) (Bourgeois & Boissinot, 2019). TEs have been divided into class I (retrotransposons), commonly called copy and paste elements, and class II (transposable DNA), or also called “cut and paste” transposable elements (B. Gao et al., 2016). Since the revelation of numerous eukaryotic TEs, for example miniature inverted repeat transposable elements (MITEs), this arrangement has been tested, as it is difficult to put the new transposable elements in the current system (Morgante et al., 2005).

Single Nucleotide Marker

SNPs are the most abundantly available molecular markers in plants, even in species that are restricted in their genetic diversity. However, for many crop plants there are surprisingly low numbers of validated SNP markers available although they are needed in large numbers for studies regarding, linkage mapping, population structure analysis, genetic variation, association genetics, map-based gene isolation, and plant breeding (Ganal et al., 2009). In cotton, initial SNP marker development has been slow and costly, and few SNP markers were made available (Van Deynze et al., 2009). there was considerable progress toward the development of new cotton genomic resources. Two genome-sequencing projects of the diploid cottons have been completed; one of the ancestral progenitors of the A genome (*G. arboreum*—A_2) and the closest relative, D genome (*G. raimondii*—D_5), to the tetraploids reported by two groups (F. Li et al., 2014).

There are many techniques for the identification of SNPs some are as follow; SNPs identification on the basis of EST sequence data. SNPs identification on the basis of array analysis. SNPs identification by using next generation sequence technologies. SNPs identification from sequenced genome. Amplicon resequencing. These markers are also associated with several desirable traits like yield, fiber quality, boll size and genes respond to biotic and abiotic stresses in cotton. Changes in yield related traits are of interest to plant breeders. Numerous quantitative trait loci with novel functions have been identified in cotton by using these markers. This information can be used for crop improvement through molecular breeding approaches (Majeed et al., 2019).

Genome Selection

The best individuals are selected on the base of their breeding value. It is more beneficial than traditional or conventional methods because it takes less time to produce new breeds. Genome wide marker is used in it to locate the gene of interest. It can be used to make connection between marker and phenotypic characters. (X. Wang et al., 2018). GS has been widely adopted in animal breeding programs globally because of its potential to improve selection accuracy, minimize phenotyping, reduce cycle time, and increase genetic gains (Budhlakoti et al., 2022).

In addition, given the promising initial evaluation outcomes of GS for the improvement of yield, biotic and abiotic stress tolerance, and quality in cereal crops like wheat, maize, and rice, prospects of integrating it in breeding crops are also being explored. In this approach, the individual effect of each marker is estimated, and the additive sum of all the marker effects is used for calculation of the genomic-estimated breeding values (GEBV) of each individual. In the current scenario of climate change, GS is a promising tool for improving the genetic gain of individuals under the breeding program (Yuan et al., 2019).

The basic process of any genomic selection process starts with the creation of training population, i.e., individuals having both genotypic and phenotypic

information, and this information is used to build a model, where the phenotype is used as a response and genotype as a predictor (Heffner et al., 2011). The choice of models is an important factor in implementing GS, and several parametric and non-parametric genomic prediction models are available for this purpose. One of the most common and widely used parametric genomic selection model is the best linear unbiased prediction (BLUP). It is a mixed model–based whole-genome regression approach that is used to estimate the marker effects, and the same has been successfully applied to predict complex traits (Habier et al., 2013).

Quantitative trait loci

A QTL is a small section of DNA on a chromosome thought to influence a specific trait. Scientists search different areas of the genome for locations (i.e., loci) they can associate with the trait. The gene included in each QTL exists in more than one form, or allele, and can differ between individuals in a population. (Copeland et al., 1993). Genetic improvement in fiber quality is one of the main challenges for cotton breeders. Quantitative trait loci (QTL) mapping provides a powerful approach to dissect the molecular mechanism in fiber quality traits (Ma et al., 2020).

Genome Editing

To accomplish goals in a short amount of time, contemporary plant breeding techniques, in particular modern genome editing technologies (GETs), can be used. Numerous crop improvement initiatives have made use of GETs, such as zinc-finger nucleases, transcription-activator-like effector nucleases, clustered regularly interspaced palindromic repeats (CRISPR), and CRISPR-associated proteins systems (CRISPR/Cas)-based technologies.

Genome editing (GenEd) has revolutionized the field of life sciences and is used for genetic engineering in plants and animals with equal success. Researchers are using GenEd technology to get precise genetic modifications (Wen et al., 2018). Utilizing GenEd technologies, a number of organisms have been genetically created for selective genetic modification e.g. *Arabidopsis thaliana* (Cermak et al., 2011), wheat (Wang et al., 2014), cotton (Li et al., 2017; Wang et al., 2018).

Zinc-finger Nucleases (ZFNs)

Zinc-finger nucleases (ZFNs) which are composed of engineered zinc-finger DNA-binding domains fused with a nuclease, generally the *Fok*I nucleases. The zinc-finger domains are composed of a series of four to six 30 amino acid domains that can bind to trinucleotide sequences giving the entire DNA-binding domain specificity to 12–18 nucleotides. Since the *Fok*I nuclease functions as a dimer, pairs of zinc-finger domains are designed to bind upstream and downstream of the cut site which increases the specificity of the complete ZFN to 24–36 nucleotides. The ability of these engineered nucleases to create targeted double-stranded breaks at designated locations throughout the genome has enabled precise deletion, addition, and editing of genes. These techniques are being used to create new genetic variation by deleting or editing endogenous gene sequences and enhancing the efficiency of transgenic product development through targeted insertion of transgenes to specific genomic locations and to sequentially add and/or delete transgenes from existing transgenic events (Davies et al., 2017).

TALENs

The use of TALEs and TALENs for precise genome modifications of plants is now a common practice. So far, tens of crop plants have been modified using engineered nucleases like rice, wheat, tomato, potato, tobacco, maize, barley, cotton, etc. The TALE and TALEN technology is being used for development of biotic and abiotic stress-resistant plants as well as yield and quality improvement (Khan et al., 2017). Transcription activator-like effector nucleases (TALENs) in particular, consisting of a free designable DNA binding domain and a nuclease, have been exploited today by a huge number of approaches in many different organisms.

The convenience of designing the DNA binding domain and straightforward protocols for their assembly, as well as the broad number of applications in different scientific fields made it Natures method of the year 2011. TALENs act as molecular scissors by introducing double strand breaks (DSBs) to the DNA at a given location. The DSBs are subsequently repaired by the cell itself using different repair pathways such as non-homologous end joining (NHEJ) or homologous recombination (HR) (Fig. 1). These mechanisms can lead to deletions, insertions, replacements or larger chromosomal rearrangements. By offering a template DNA it is possible to channel the repair in direction of HR (Sprink et al., 2015).

CRISPR Cas-9

CRISPR/Cas9 system is emerging as effective strategy for generating site-specific mutations. Recently, CRISPR/Cas9-mediated genome editing system have been rapidly optimized and applied in crop genetic improvement (Table 1) (Li & Zhang, 2019).

Table 1: The traits improved in different crops as a result of genome editing techniques

Traits	Organism	References
1st experimental information of CRISPR Cas mechanism action	Bacteria *Streptococcus thermophilus*	(Barrangou et al., 2007)
Disease resistance	Apple	(Pompili et al., 2020)
Height/ flower timing	Maize	(Q. Li et al., 2020)
Grain size salt tolerance	Rice	(Usman et al., 2021)
Flowering timing	Soybean	(Cai et al., 2020)
Analysis of a novel ethylene responsive transcription factor gene *GhERF4*	Cotton	(Jin & Liu, 2008)
Vacuolar-invertase genes were identified and sequenced	Cotton	(Taliercio et al., 2010)
Silencing GhNDR1 and GhMKK2 compromises cotton resistance to Verticillium wilt	Cotton	(Gao et al., 2011)
Increased lateral root formation, editing of arginase genes	Cotton	(Wang et al., 2017)
Suppression of Cotton Leaf Curl Virus	Cotton	(Binyameen et al., 2021)

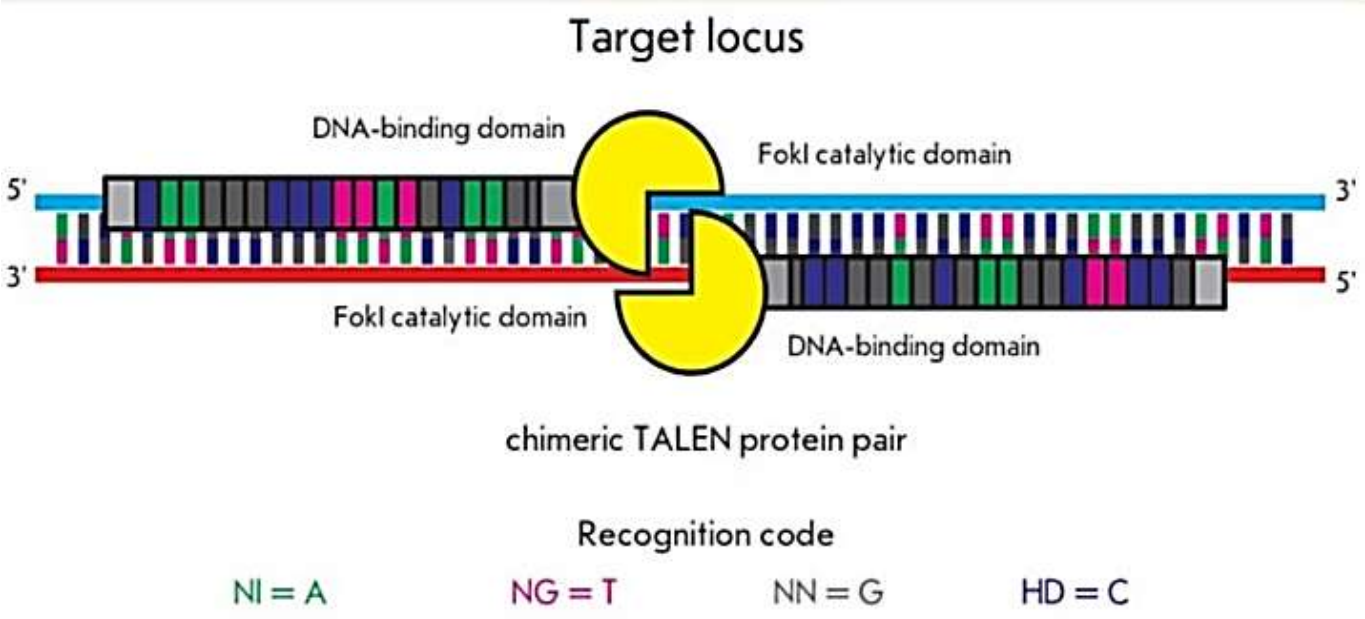

Fig. 1: A scheme for introducing a double-strand break using chimeric TALEN proteins (Nemudryi et al., 2014).

Cas9 has proved to be versatile technique that has very recently been deployed successfully to control different Gemini viruses. The CRISPR/Cas9 system has been proved to be a comprehensive technique to control different Gemini viruses, however, like previously used techniques, only a single virus is targeted and it has not been deployed to control begomovirus complexes associated with DNA-satellites (Iqbal et al., 2016).

In bacteria and archaea, the CRISPR/Cas9 system serves as the RNA-based adaptive immune system. *Streptococcus pyogenes* is the source of the type II CRISPR system, which includes CRISPR-associated nuclease 9 (Cas9) (Zuo et al., 2022).

In cotton, the CRISPR/Cas system can help to enhance biotic and abiotic stress resistance, modify gene expression, and gene stacking of important traits with minimum chances of segregation. The transgene clean approach further enhances CRISPR acceptability, and plants can further utilized for selfing or backcrossing to improve traits under investigation (Fiaz et al., 2021).

CRISPR-based genome editing has revolutionized the practice of plant breeding by providing a more precise, cost-efficient, and rapid tool for creating desirable traits in plants. This technology has enabled the transfer of beneficial traits from one species to another, while minimizing or eliminating undesirable traits (Nerkar et al., 2022). This system has also been used to develop new varieties of crops that are more resistant to diseases and (Zaidi et al., 2020) environmental stress, (Kouhen et al., 2023) and that has improved nutritional profiles (Kumar et al., 2022).

CRISPR/Cas systems can be designed by inserting the DNA target protospacer sequence into the crRNAs or sgRNAs. The editing potential of these tools has increased as a result of the discovery of several PAM (protospacer adjacent motif) specific Cas orthologs & polymorphisms (Anzalone et al., 2020). With this technique, foreign nucleic acids are specifically interfered with based on the sequence of short guide RNAs. Target locus needs alteration of genome *via* CRISPR/Cas9. DSBs, which happen when two repair processes alter the same gene, are brought

on by the site-specific nucleases. Genes are deleted or fused using NHEJ, or non-homologous ending combining, is carried out without donor DNA. By using homologous portions as its foundation, homology-directed repair (HDR) adjusts gene sequences in response to even the smallest changes in either DNA strand.

The targeting sequence (crRNA), which is situated 20 nucleotides before the PAM sequence, will be divided into roughly three bases by the Cas9 nuclease. The target region's gRNA can only attach to the genomic DNA if it has a particular protospacer neighboring motif (PAM). Later, the Cas9 nuclease separates the DNA into two strands (denoted by the scissors). A customized sgRNA with a Cas9 nuclease-recruiting domain and an aiming sequence (crRNA sequence) is required by the CRISPR/Cas9 system (tra crRNA) (Saini et al., 2023). The mechanism of CRISPR/Cas-9 genome editing can be generally divided into three steps: recognition, cleavage, and repair (Shao et al., 2016). The designed sgRNA directs Cas-9 and recognizes the target sequence in the gene of interest through its 5'crRNA complementary base pair component. The Cas-9 protein remains inactive in the absence of sgRNA. The Cas-9 nuclease makes double-stranded breaks (DSBs) at a site 3 base pair upstream to PAM (Ceasar et al., 2016).

The most commonly used nuclease in the genome-editing tool, Cas-9 protein recognizes the PAM sequence at 5'-NGG-3' (N can be any nucleotide base). Once Cas-9 has found a target site with the appropriate PAM, it triggers local DNA melting followed by the formation of RNA-DNA hybrid (Fig. 2). Then, the Cas-9 protein is activated for DNA cleavage. HNH domain cleaves the complementary strand, while the RuvC domain cleaves the non-complementary strand of target DNA to produce predominantly blunt-ended DSBs. Finally, the DSB is repaired by the host cellular machinery (Jiang & Doudna, 2017; Mei et al., 2016).

CRISPR Cas in crop Quality Improvement

Crop quality has played a pivotal role in determining the market value of crops. In general, crop quality is determined by external and internal traits. The external quality attributes include physical and aesthetic characteristics, such as size, color, texture, and fragrance. In contrast, the internal quality factors include nutrients (like protein, starch, lipids etc.) and bioactive compounds (such as carotenoids, lycopene, γ-aminobutyric acid, flavonoid and so on). CRISPR/Cas9-mediated crop quality improvement focused on the physical appearance, edible quality, fruit texture and nutritional value. (Q. Liu et al., 2021).

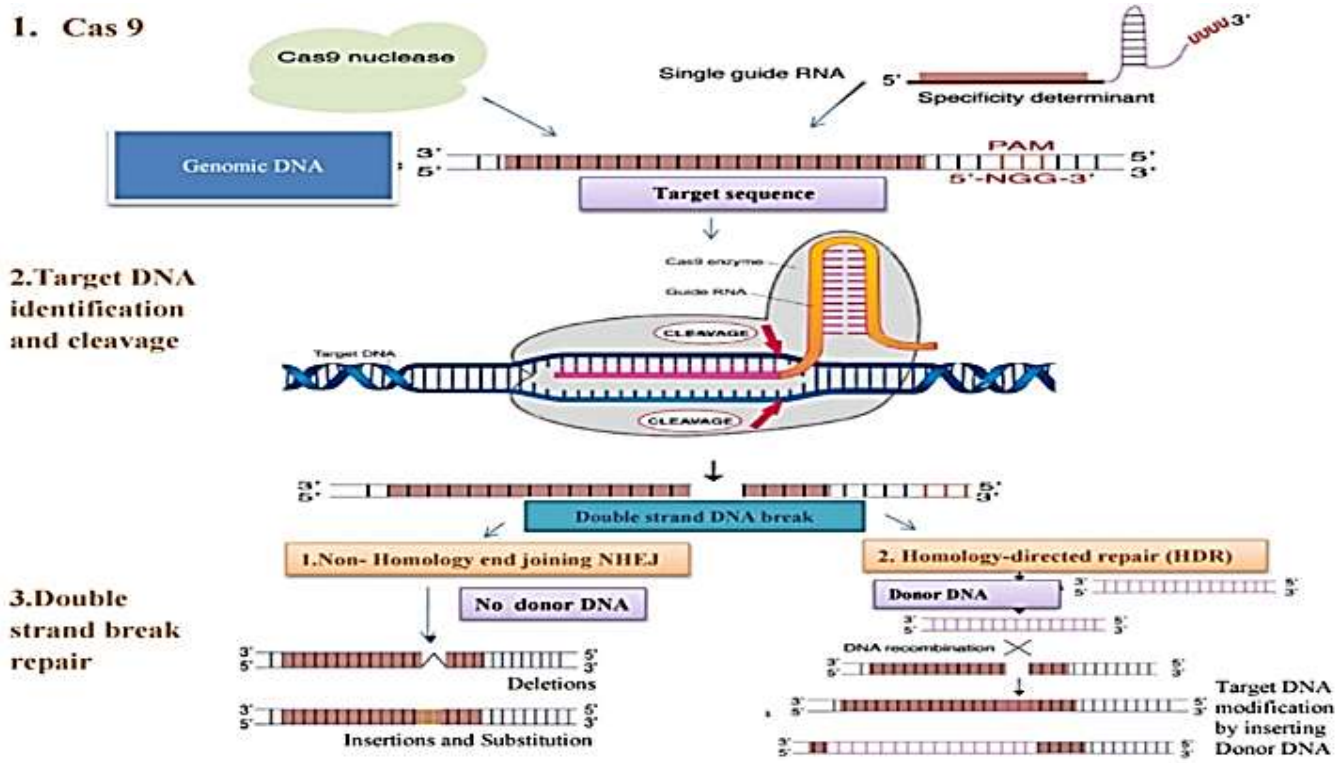

Fig. 2: Overview of CRISPR/Cas9 technology for plant genome editing (Saini *et al*., 2023).

Through using CRISPR/Cas9 system, a single dominant *Waxy* gene controlling amylose content was

knocked out in two rice varieties, and the resulting mutants showed low amylose levels and elevated glutinosity. This research provides a simple and successful method for transforming a low-quality rice variety into a higher-quality one. Furthermore, the *GBSS* gene, which encodes a granule-bound starch synthase, was damaged in tetraploid potato using CRISPR/Cas9 (Lei et al., 2021).

(Gate et al., 2024) concluded that Germplasm enhancement, a lack of cotton seed production, and abiotic and biotic stresses are major production and cotton breeding constraints. Breeders are currently experimenting with these factors. Cotton breeding in Ethiopia has achieved varietal development. Among those released varieties, the major ones include disease resistance, high yield, pest resistance, high fiber quality, comfort to mechanical harvesting by increasing cotton height, early maturity and adaptability to harsh environments.

Components of CRISPR Cas

Based on the structure and functions of Cas-proteins, CRISPR/Cas system can be divided into Class I (type I, III, and IV) and Class II (type II, V, and VI). The class I systems consist of multi-subunit Cas-protein complexes, while the class II systems utilize a single Cas-protein. Since the structure of type II CRISPR/Cas-9 is relatively simple, it has been well studied and extensively used in genetic engineering (Z. Liu et al., 2020).

Guide RNA (gRNA) and CRISPR-associated (Cas-9) proteins are the two essential components in CRISPR/Cas-9 system. The Cas-9 protein, the first Cas protein used in genome editing was extracted from *Streptococcus pyogenes* (SpCas-9). It is a large (1368 amino acids) multi-domain DNA endonuclease responsible for cleaving the target DNA to form a double-stranded break and is called a genetic scissor. Guide RNA is made up of two parts, CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tra crRNA). The crRNA is an 18–20 base pair in length that specifies the target DNA by pairing with the target sequence, whereas tra crRNA is a long stretch of loops that serve as a binding scaffold for Cas-9 nuclease (Mei et al., 2016).

REFERENCES

Anand, A., Subramanian, M., & Kar, D. (2023). Breeding techniques to dispense higher genetic gains. *Frontiers in Plant Science*, *13*, 1076094. https://doi.org/10.3389/fpls.2022.1076094

Anzalone, A. V., Koblan, L. W., & Liu, D. R. (2020). Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. *Nature Biotechnology*, *38*(7), 824–844. https://doi.org/10.1038/s41587-020-0561-9

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes. *Science*, *315*(5819), 1709–1712. https://doi.org/10.1126/science.1138140

Bhargava, A., & Srivastava, S. (2019). Plant Breeding. In A. Bhargava & S. Srivastava, *Participatory Plant Breeding: Concept and Applications* (pp. 29–68). Springer Singapore. https://doi.org/10.1007/978-981-13-7119-6_2

Binyameen, B., Khan, Z., Khan, S. H., Ahmad, A., Munawar, N., Mubarik, M. S., Riaz, H., Ali, Z., Khan, A. A., Qusmani, A. T., Abd-Elsalam, K. A., & Qari, S. H. (2021). Using Multiplexed CRISPR/Cas9 for Suppression of Cotton Leaf Curl Virus. *International Journal of Molecular Sciences*, *22*(22), 12543. https://doi.org/10.3390/ijms222212543

Bourgeois, Y., & Boissinot, S. (2019). On the Population Dynamics of Junk: A Review on the Population Genomics of Transposable Elements. *Genes*, *10*(6), 419. https://doi.org/10.3390/genes10060419

Bourland, F., & Myers, G. O. (2015). Conventional Cotton Breeding. In D. D. Fang & R. G. Percy (Eds.), *Agronomy Monographs* (pp. 205–228). American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. https://doi.org/10.2134/agronmonogr57.2013.0025

Budhlakoti, N., Kushwaha, A. K., Rai, A., Chaturvedi, K. K., Kumar, A., Pradhan, A. K., Kumar, U., Kumar, R. R., Juliana, P., Mishra, D. C., & Kumar, S. (2022). Genomic Selection: A Tool for Accelerating the Efficiency of Molecular Breeding for Development of Climate-Resilient Crops. *Frontiers in Genetics*, *13*, 832153. https://doi.org/10.3389/fgene.2022.832153

Cai, Y., Wang, L., Chen, L., Wu, T., Liu, L., Sun, S., Wu, C., Yao, W., Jiang, B., Yuan, S., Han, T., & Hou, W. (2020). Mutagenesis of *GmFT2a* and *GmFT5a* mediated by CRISPR/Cas9 contributes for expanding the regional adaptability of soybean. *Plant Biotechnology Journal*, *18*(1), 298–309. https://doi.org/10.1111/pbi.13199

Ceasar, S. A., Rajan, V., Prykhozhij, S. V., Berman, J. N., & Ignacimuthu, S. (2016). Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1863*(9), 2333–2344. https://doi.org/10.1016/j.bbamcr.2016.06.009

Cermak, T., Doyle, E. L., Christian, M., Wang, L., Zhang, Y., Schmidt, C., Baller, J. A., Somia, N. V., Bogdanove, A. J., & Voytas, D. F. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Research*, *39*(12), e82–e82. https://doi.org/10.1093/nar/gkr218

Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *363*(1491), 557–572. https://doi.org/10.1098/rstb.2007.2170

Constable, G. A., & Bange, M. P. (2015). The yield potential of cotton (*Gossypium hirsutum* L.). *Field Crops Research*, *182*, 98–106. https://doi.org/10.1016/j.fcr.2015.07.017

Copeland, N. G., Jenkins, N. A., Gilbert, D. J., Eppig, J. T., Maltais, L. J., Miller, J. C., Dietrich, W. F., Weaver, A., Lincoln, S. E., Steen, R. G., Stein, L. D., Nadeau, J. H., & Lander, E. S. (1993). A Genetic Linkage Map of the

Mouse: Current Applications and Future Prospects. *Science*, *262*(5130), 57–66. https://doi.org/10.1126/science.8211130

Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquín, D., De Los Campos, G., Burgueño, J., González-Camacho, J. M., Pérez-Elizalde, S., Beyene, Y., Dreisigacker, S., Singh, R., Zhang, X., Gowda, M., Roorkiwal, M., Rutkoski, J., & Varshney, R. K. (2017). Genomic Selection in Plant Breeding: Methods, Models, and Perspectives. *Trends in Plant Science*, *22*(11), 961–975. https://doi.org/10.1016/j.tplants.2017.08.011

Davies, J. P., Kumar, S., & Sastry-Dent, L. (2017). Use of Zinc-Finger Nucleases for Crop Improvement. In *Progress in Molecular Biology and Translational Science* (Vol. 149, pp. 47–63). Elsevier. https://doi.org/10.1016/bs.pmbts.2017.03.006

Deshmukh, R. (2021). Impact of GM Crops on Agriculture Production: A Positive Reflection through Success Stories. In *Policy Issues in Genetically Modified Crops* (pp. 161–187). Elsevier. https://doi.org/10.1016/B978-0-12-820780-2.00008-X

Fiaz, S., Khan, S. A., Younas, A., Shahzad, K., Ali, H., Noor, M. A., Ashraf, U., & Nadeem, F. (2021). Application of CRISPR/Cas system for genome editing in cotton. In *CRISPR and RNAi Systems* (pp. 277–301). Elsevier. https://doi.org/10.1016/B978-0-12-821910-2.00010-2

Freeland, J. R. (2017). The importance of molecular markers and primer design when characterizing biodiversity from environmental DNA. *Genome*, *60*(4), 358–374. https://doi.org/10.1139/gen-2016-0100

Fu, Y.-B. (2015). Understanding crop genetic diversity under modern plant breeding. *Theoretical and Applied Genetics*, *128*(11), 2131–2142. https://doi.org/10.1007/s00122-015-2585-y

Ganal, M. W., Altmann, T., & Röder, M. S. (2009). SNP identification in crop plants. *Current Opinion in Plant Biology*, *12*(2), 211–217. https://doi.org/10.1016/j.pbi.2008.12.009

Gao, B., Shen, D., Xue, S., Chen, C., Cui, H., & Song, C. (2016). The contribution of transposable elements to size variations between four teleost genomes. *Mobile DNA*, *7*(1), 4. https://doi.org/10.1186/s13100-016-0059-7

Gao, X., Wheeler, T., Li, Z., Kenerley, C. M., He, P., & Shan, L. (2011). Silencing *GhNDR1* and *GhMKK2* compromises cotton resistance to Verticillium wilt. *The Plant Journal*, *66*(2), 293–305. https://doi.org/10.1111/j.1365-313X.2011.04491.x

Gate, A., Abate, A., & Semahagn, B. K. (2024). Cotton Genetics and Breeding. In K. Murugesh Babu, A. K. Kabish, G. B. Tesema, & B. K. Semahagn (Eds.), *Cotton Sector Development in Ethiopia* (pp. 19–38). Springer Nature Singapore. https://doi.org/10.1007/978-981-99-9149-5_2

Guzman, M. A., Vilain, L. A., Rondon, T. M., & Sanchez, J. (2021). Genetic gain in lint yield and its components of upland cotton released during 1963 to 2010 in Venezuela. *Crop Science*, *61*(5), 3436–3444. https://doi.org/10.1002/csc2.20547

Habier, D., Fernando, R. L., & Garrick, D. J. (2013). Genomic BLUP Decoded: A Look into the Black Box of Genomic Prediction. *Genetics*, *194*(3), 597–607. https://doi.org/10.1534/genetics.113.152207

Heffner, E. L., Jannink, J., & Sorrells, M. E. (2011). Genomic Selection Accuracy using Multifamily Prediction Models in a Wheat Breeding Program. *The Plant Genome*, *4*(1), plantgenome2010.12.0029. https://doi.org/10.3835/plantgenome2010.12.0029

Hu, G., Grover, C. E., Jareczek, J., Yuan, D., Dong, Y., Miller, E., Conover, J. L., & Wendel, J. F. (2022). Correction to: Evolution and Diversity of the Cotton Genome. In M.-Rahman, Y. Zafar, & T. Zhang (Eds.), *Cotton Precision Breeding* (pp. C1–C1). Springer International Publishing. https://doi.org/10.1007/978-3-030-64504-5_18

Hu, G., & Wendel, J. F. (2019). *Cis – trans* controls and regulatory novelty accompanying allopolyploidization. *New Phytologist*, *221*(4), 1691–1700. https://doi.org/10.1111/nph.15515

Huang, G., Huang, J.-Q., Chen, X.-Y., & Zhu, Y.-X. (2021). Recent Advances and Future Perspectives in Cotton Research. *Annual Review of Plant Biology*, *72*(Volume 72, 2021), 437–462. https://doi.org/10.1146/annurev-arplant-080720-113241

Huang, G., & Zhu, Y. (2019). Plant polyploidy and evolution. *Journal of Integrative Plant Biology*, *61*(1), 4–6. https://doi.org/10.1111/jipb.12758

Iqbal, Z., Sattar, M. N., & Shafiq, M. (2016). CRISPR/Cas9: A Tool to Circumscribe Cotton Leaf Curl Disease. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00475

Islam, M. S., Fang, D. D., Jenkins, J. N., Guo, J., McCarty, J. C., & Jones, D. C. (2020). Evaluation of genomic selection methods for predicting fiber quality traits in Upland cotton. *Molecular Genetics and Genomics*, *295*(1), 67–79. https://doi.org/10.1007/s00438-019-01599-z

Jiang, F., & Doudna, J. A. (2017). CRISPR–Cas9 Structures and Mechanisms. *Annual Review of Biophysics*, *46*(1), 505–529. https://doi.org/10.1146/annurev-biophys-062215-010822

Jin, L.-G., & Liu, J.-Y. (2008). Molecular cloning, expression profile and promoter analysis of a novel ethylene responsive transcription factor gene GhERF4 from cotton (Gossypium hirstum). *Plant Physiology and Biochemistry*, *46*(1), 46–53. https://doi.org/10.1016/j.plaphy.2007.10.004

Khadi, B. M., Santhy, V., & yadav, M. S. (2010). Cotton: An introduction. *Biotechnological Advances*, 1–14.

Khan, Z., Khan, S. H., Mubarik, M. S., Sadia, B., & Ahmad, A. (2017). Use of TALEs and TALEN Technology for Genetic Improvement of Plants. *Plant Molecular Biology Reporter*, *35*(1), 1–19. https://doi.org/10.1007/s11105-016-0997-8

Knight, R. L. (1945). *Knight, R. L. (1945). The theory and application of the backcross technique in cotton breeding. Journal of Genetics, 47(1), 76-86.*

Koebner, R. M. D., & Summers, R. W. (2003). 21st century wheat breeding: Plot selection or plate detection? *Trends in Biotechnology*, *21*(2), 59–63. https://doi.org/10.1016/S0167-7799(02)00036-7

Kouhen, M., García-Caparrós, P., Twyman, R. M., Abdelly, C., Mahmoudi, H., Schillberg, S., & Debez, A. (2023). Improving environmental stress resilience in crops by genome editing: Insights from extremophile plants. *Critical Reviews in Biotechnology*, *43*(4), 559–574. https://doi.org/10.1080/07388551.2022.2042481

Kumar, D., Yadav, A., Ahmad, R., Dwivedi, U. N., & Yadav, K. (2022). CRISPR-Based Genome Editing for Nutrient Enrichment in Crops: A Promising Approach Toward

Global Food Security. *Frontiers in Genetics*, *13*, 932859. https://doi.org/10.3389/fgene.2022.932859

Lei, J., Dai, P., Li, J., Yang, M., Li, X., Zhang, W., Zhou, G., WangzhenGuo, & Liu, X. (2021). Tissue-Specific CRISPR/Cas9 System of Cotton Pollen with GhPLIMP2b and GhMYB24 Promoters. *Journal of Plant Biology*, *64*(1), 13–21. https://doi.org/10.1007/s12374-020-09272-4

Li, C., Unver, T., & Zhang, B. (2017). A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in Cotton (Gossypium hirsutum L.). *Scientific Reports*, *7*(1), 43902. https://doi.org/10.1038/srep43902

Li, C., & Zhang, B. (2019). Genome Editing in Cotton Using CRISPR/Cas9 System. In B. Zhang (Ed.), *Transgenic Cotton* (Vol. 1902, pp. 95–104). Springer New York. https://doi.org/10.1007/978-1-4939-8952-2_8

Li, F., Fan, G., Wang, K., Sun, F., Yuan, Y., Song, G., Li, Q., Ma, Z., Lu, C., Zou, C., Chen, W., Liang, X., Shang, H., Liu, W., Shi, C., Xiao, G., Gou, C., Ye, W., Xu, X., … Yu, S. (2014). Genome sequence of the cultivated cotton Gossypium arboreum. *Nature Genetics*, *46*(6), 567–572. https://doi.org/10.1038/ng.2987

Li, Q., Lu, L., Liu, H., Bai, X., Zhou, X., Wu, B., Yuan, M., Yang, L., & Xing, Y. (2020). A minor QTL, SG3, encoding an R2R3-MYB protein, negatively controls grain length in rice. *Theoretical and Applied Genetics*, *133*(8), 2387–2399. https://doi.org/10.1007/s00122-020-03606-z

Liu, Q., Yang, F., Zhang, J., Liu, H., Rahman, S., Islam, S., Ma, W., & She, M. (2021). Application of CRISPR/Cas9 in Crop Quality Improvement. *International Journal of Molecular Sciences*, *22*(8), 4206. https://doi.org/10.3390/ijms22084206

Liu, Z., Dong, H., Cui, Y., Cong, L., & Zhang, D. (2020). Application of different types of CRISPR/Cas-based systems in bacteria. *Microbial Cell Factories*, *19*(1), 172. https://doi.org/10.1186/s12934-020-01431-z

Ma, L., Su, Y., Nie, H., Cui, Y., Cheng, C., Ijaz, B., & Hua, J. (2020). QTL and genetic analysis controlling fiber quality traits using paternal backcross population in upland cotton. *Journal of Cotton Research*, *3*(1), 22. https://doi.org/10.1186/s42397-020-00060-6

Mackill, D. J., Nguyen, H. T., & Zhang, J. (1999). Use of molecular markers in plant improvement programs for rainfed lowland rice. *Field Crops Research*, *64*(1–2), 177–185. https://doi.org/10.1016/S0378-4290(99)00058-1

Majeed, S., Rana, I. A., Atif, R. M., Ali, Z., Hinze, L., & Azhar, M. T. (2019). Role of SNPs in determining QTLs for major traits in cotton. *Journal of Cotton Research*, *2*(1), 5. https://doi.org/10.1186/s42397-019-0022-5

Mei, Y., Wang, Y., Chen, H., Sun, Z. S., & Ju, X.-D. (2016). Recent Progress in CRISPR/Cas9 Technology. *Journal of Genetics and Genomics*, *43*(2), 63–75. https://doi.org/10.1016/j.jgg.2016.01.001

Morgante, M., Brunner, S., Pea, G., Fengler, K., Zuccolo, A., & Rafalski, A. (2005). Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in maize. *Nature Genetics*, *37*(9), 997–1002. https://doi.org/10.1038/ng1615

Negm, M. (2020). Cotton breeding. In *Handbook of Natural Fibres* (pp. 579–603). Elsevier. https://doi.org/10.1016/B978-0-12-818398-4.00018-9

Nerkar, G., Devarumath, S., Purankar, M., Kumar, A., Valarmathi, R., Devarumath, R., & Appunu, C. (2022). Advances in Crop Breeding Through Precision Genome Editing. *Frontiers in Genetics*, *13*, 880195. https://doi.org/10.3389/fgene.2022.880195

Niles, G. A., & Feaster, C. V. (2016). Breeding. In R. J. Kohel & C. F. Lewis (Eds.), *Agronomy Monographs* (pp. 201–231). American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. https://doi.org/10.2134/agronmonogr24.c7

Orton, T. J. (2020). Introduction. In *Horticultural Plant Breeding* (pp. 3–7). Elsevier. https://doi.org/10.1016/B978-0-12-815396-3.09986-8

Paterson, A. H. (Ed.). (2009). *Genetics and Genomics of Cotton*. Springer US. https://doi.org/10.1007/978-0-387-70810-2

Pompili, V., Dalla Costa, L., Piazza, S., Pindo, M., & Malnoy, M. (2020). Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system. *Plant Biotechnology Journal*, *18*(3), 845–858. https://doi.org/10.1111/pbi.13253

Saini, H., Thakur, R., Gill, R., Tyagi, K., & Goswami, M. (2023). CRISPR/Cas9-gene editing approaches in plant breeding. *GM Crops & Food*, *14*(1), 1–17. https://doi.org/10.1080/21645698.2023.2256930

Shao, M., Xu, T.-R., & Chen, C.-S. (2016). The big bang of genome editing technology: Development and application of the CRISPR/Cas9 system in disease animal models. *Dong Wu Xue Yan Jiu = Zoological Research*, *37*(4), 191–204. https://doi.org/10.13918/j.issn.2095-8137.2016.4.191

Sprink, T., Metje, J., & Hartung, F. (2015). Plant genome editing by novel tools: TALEN and other sequence specific nucleases. *Current Opinion in Biotechnology*, *32*, 47–53. https://doi.org/10.1016/j.copbio.2014.11.010

Taliercio, E., Scheffler, J., & Scheffler, B. (2010). Characterization of two cotton (Gossypium hirsutum L) invertase genes. *Molecular Biology Reports*, *37*(8), 3915–3920. https://doi.org/10.1007/s11033-010-0048-8

Usman, B., Zhao, N., Nawaz, G., Qin, B., Liu, F., Liu, Y., & Li, R. (2021). CRISPR/Cas9 Guided Mutagenesis of Grain Size 3 Confers Increased Rice (Oryza sativa L.) Grain Length by Regulating Cysteine Proteinase Inhibitor and Ubiquitin-Related Proteins. *International Journal of Molecular Sciences*, *22*(6), 3225. https://doi.org/10.3390/ijms22063225

Van Deynze, A., Stoffel, K., Lee, M., Wilkins, T. A., Kozik, A., Cantrell, R. G., Yu, J. Z., Kohel, R. J., & Stelly, D. M. (2009). Sampling nucleotide diversity in cotton. *BMC Plant Biology*, *9*(1), 125. https://doi.org/10.1186/1471-2229-9-125

Wang, P., Zhang, J., Sun, L., Ma, Y., Xu, J., Liang, S., Deng, J., Tan, J., Zhang, Q., Tu, L., Daniell, H., Jin, S., & Zhang, X. (2018). High efficient multisites genome editing in allotetraploid cotton (*Gossypium hirsutum*) using CRISPR/Cas9 system. *Plant Biotechnology Journal*, *16*(1), 137–150. https://doi.org/10.1111/pbi.12755

Wang, X., Xu, Y., Hu, Z., & Xu, C. (2018). Genomic selection methods for crop improvement: Current status and prospects. *The Crop Journal*, *6*(4), 330–340. https://doi.org/10.1016/j.cj.2018.03.001

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J.-L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, *32*(9), 947–951. https://doi.org/10.1038/nbt.2969

Wang, Y., Meng, Z., Liang, C., Meng, Z., Wang, Y., Sun, G., Zhu, T., Cai, Y., Guo, S., Zhang, R., & Lin, Y. (2017). Increased lateral root formation by CRISPR/Cas9-mediated editing of arginase genes in cotton. *Science China Life Sciences*, *60*(5), 524–527. https://doi.org/10.1007/s11427-017-9031-y

Wen, S., Liu, H., Li, X., Chen, X., Hong, Y., Li, H., Lu, Q., & Liang, X. (2018). TALEN-mediated targeted mutagenesis of fatty acid desaturase 2 (FAD2) in peanut (Arachis hypogaea L.) promotes the accumulation of oleic acid. *Plant Molecular Biology*, *97*(1–2), 177–185. https://doi.org/10.1007/s11103-018-0731-z

Yali, W., & Mitiku, T. (2022a). Mutation Breeding and Its Importance in Modern Plant Breeding. *Journal of Plant Sciences*, *10*(2), 64. https://doi.org/10.11648/j.jps.20221002.13

Yali, W., & Mitiku, T. (2022b). Mutation Breeding and Its Importance in Modern Plant Breeding. *Journal of Plant Sciences*, *10*(2), 64. https://doi.org/10.11648/j.jps.20221002.13

Yang, H.-B., Kang, W.-H., Nahm, S.-H., & Kang, B.-C. (2015). Methods for Developing Molecular Markers. In H.-J. Koh, S.-Y. Kwon, & M. Thomson (Eds.), *Current Technologies in Plant Molecular Breeding* (pp. 15–50). Springer Netherlands. https://doi.org/10.1007/978-94-017-9996-6_2

Yuan, Y., Cairns, J. E., Babu, R., Gowda, M., Makumbi, D., Magorokosho, C., Zhang, A., Liu, Y., Wang, N., Hao, Z., San Vicente, F., Olsen, M. S., Prasanna, B. M., Lu, Y., & Zhang, X. (2019). Genome-Wide Association Mapping and Genomic Prediction Analyses Reveal the Genetic Architecture of Grain Yield and Flowering Time Under Drought and Heat Stress Conditions in Maize. *Frontiers in Plant Science*, *9*, 1919. https://doi.org/10.3389/fpls.2018.01919

Zaidi, S. S.-A., Mahas, A., Vanderschuren, H., & Mahfouz, M. M. (2020). Engineering crops of the future: CRISPR approaches to develop climate-resilient and disease-resistant plants. *Genome Biology*, *21*(1), 289. https://doi.org/10.1186/s13059-020-02204-y

Zuo, Z., Babu, K., Ganguly, C., Zolekar, A., Newsom, S., Rajan, R., Wang, Y.-C., & Liu, J. (2022). Rational Engineering of CRISPR-Cas9 Nuclease to Attenuate Position-Dependent Off-Target Effects. *The CRISPR Journal*, *5*(2), 329–340. https://doi.org/10.1089/crispr.2021.0076

Nemudryi, A. A., Valetdinova, K. R., Medvedev, S. P., & Zakian, S. Á. (2014). TALEN and CRISPR/Cas genome editing systems: tools of discovery. Acta Naturae (англоязычная версия), 6(3 (22)), 19-40.

Saini, H., Thakur, R., Gill, R., Tyagi, K., & Goswami, M. (2023). CRISPR/Cas9-gene editing approaches in plant breeding. GM crops & food, 14(1), 1-17.