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REVIEW ARTICLE

Genome editing for biotic and abiotic stress management in banana: A comprehensive review

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Abstract

Bananas, including plantains (*Musa* spp.), are among the most widely cultivated fruit crops globally. However, various biotic and abiotic stresses hamper their production. The triploid chromosomal nature of most cultivated banana varieties poses significant challenges to conventional breeding efforts. Gene editing has recently emerged as a powerful tool to address these challenges. Among available technologies, CRISPR/Cas9 stands out for its precision, efficiency and relatively short development time. The CRISPR/Cas9 system operates through an RNA-guided endonuclease mechanism that introduces double-strand breaks (DSBs) at specific genomic locations. These targeted modifications result in heritable changes, making it a promising approach for developing stress-resistant banana varieties. CRISPR/Cas9 has been employed to manage biotic stress by combating bacterial diseases such as Xanthomonas Wilt (BXW) and viral infections including Banana Streak Virus (BSV) and Banana Bunchy Top Virus (BBTV). This involves editing susceptibility genes like Musa DMR6, or enhancing the expression of defense-related genes such as chitinase. For abiotic stress tolerance, genome editing and gene overexpression techniques have been utilized to increase resilience to environmental factors like drought, salinity and cold. Additionally, disruption of the 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) gene crucial in ethylene biosynthesis has been used to reduce ethylene production, thereby extending the shelf life of banana fruits. This review explores the potential of CRISPR/Cas9 and related gene editing technologies as transformative tools in improving stress tolerance in bananas, ultimately contributing to sustainable cultivation and global food security.

Keywords: abiotic stress; 1-aminocyclopropane-1-carboxylic acid oxidase; aquaporins, banana, biotic stress, CRISPR/Cas9

Introduction

Musa spp., comprising dessert bananas and plantains, is widely cultivated in tropics and subtropical regions. Banana is one of the oldest domesticated crops and is highly valued for its nutritional content. Almost all parts of the banana fruit, flower bud, leaves and pseudo stem are utilized. The currently cultivated edible bananas are derived from two diploid species native to Southeast Asia: *Musa acuminata* (AA) and *Musa balbisiana* (BB) (1). Bananas are the world's second preferred fruit crop, with 1200 varieties (2). India dominates the world in banana production, while China, Indonesia and Brazil following closely behind. In India, bananas are cultivated on 995 million hectares, producing 37474 million metric tons of production and 37.66 MT of productivity, according to the country's second advance estimate for 2023 and 2024 With an expected 3895.64 million MT, or 10.41 %, of the nation's total banana production, Tamil Nadu ranks fourth in regards of production (3).

In India, smallholder farmers for domestic consumption and local markets primarily undertake banana cultivation. Various breeding programs across the globe have produced more than 100 cultivars. Commercially, the Cavendish group (AAA) is the most widely cultivated for dessert purposes. Other dessert banana cultivars such as Pisang Lilin (AA), Champa Nasik (AAAA), Goldfinger (AAAB), Poovan (AAB) and Figo (ABB) are grown on a smaller scale (2, 3). Cultivars such as Intuntu (AAA) and Mujuba (AAA) in East African highlands and the cultivars such as Bluggoe (ABB), Butobe (ABB), Klue Terapod (ABBB) in the Philippines and America are mainly grown as plantains types (4).

The current edible bananas are triploids (2n = 3x = 33), primarily with an AAA genome and are classified as sweet dessert bananas (1). Triploid plants are more robust and easier to cultivate than diploids offering benefits to the growers. The major advantage of growing triploids is vegetative propagation, which assures them genetic homogeneity and easy management for the growers. Because of genetic uniformity, the triploid banana plants are easily susceptible to diseases. In triploids, the sterility of clones is considered a major disadvantage for genetic improvement (5).

Banana production is severely affected by a range of biotic and abiotic stressors. Additional challenges include declining soil fertility, soil-borne pathogens and poor-quality planting material. Among these, disease and pest outbreaks are the most critical constraints on yield and productivity (6). The intensive use of agrochemicals is one of the limiting factors affecting the yield in mono cropping. The narrow genetic base and non-availability of germplasm in banana leads to erosion of varieties. The development of banana cultivars by conventional breeding methods is challenging because of narrow gene flow in Musa spp., restitution in male gametes, lengthy breeding cycles, polyploidy and sterility in most cultivars (7).

To overcome the limitations of conventional breeding, modern genetic tools such as gene editing offer promising alternatives. Gene editing enables the development of cultivars that are resistant to diseases and pests, tolerant to environmental stresses and enriched in nutritional traits. This review highlights recent advances in gene editing technologies for banana improvement.

Gene editing tools in plants

Zinc finger nucleases (ZFNs)

The discovery of eukaryotic zinc finger proteins marked a significant advancement in gene-editing technologies. Zinc Finger Nucleases (ZFNs) are chimeric proteins composed of DNA-binding zinc finger domains linked to a non-specific FokI nuclease domain (8). One zinc finger nuclease recognizes approximately three Base pairs of DNA. ZFNs are designed in such a way that they may attach and break any type of DNA sequence there by creating a DNA double stranded break (DSBs) (9). The catalytic region of the type II restriction enzyme FokI has been employed to break down DNA (10). The catalytic region must dimerize to break the DNA; therefore, two neighbouring ZFN pairs need to align themselves with proper distance at the target location. ZFNs can target 18–21 base-pair sequences, offering relatively high specificity. However, their complex protein design and potential off-target effects have limited their broader application (11).

Transcription activator-like effector nucleases (TALENs)

Xanthomonas bacteria naturally produce Transcription Activator -Like Effectors (TALEs), from which synthetic enzymes known as Transcription Activator-Like Effector Nucleases (TALENs) are derived (12). Like ZFNs, TALENs consist of a DNA-binding domain fused to a FokI nuclease domain (13). Unlike ZNFs, TALENs do not require any PAM sequence, it targets any sequence. TALENs can target methylated DNA in some contexts, but efficiency is reduced (14). ZFNs and TALENs play a significant role in gene editing, but their difficulty in cloning and protein engineering has prevented their wide use among researchers (15).

Meganucleases

In the 1980s, researchers identified two proteins from mobile genetic elements in yeast that possessed endonucleolytic properties, enabling them to induce homologous recombination (16). The remarkable feature of these proteins were sequence selectivity and capacity to break DNA at a specific, precise spot within the yeast genome (17). These two proteins can recognize 18 Base pairs DNA sequences. The word “Mega nuclease” was coined since this sequence was by far the biggest DNA sequence ever identified by any endonuclease (18). Despite their precision, meganucleases have two major limitations: first, the unique and complex DNA recognition sequence makes it difficult to engineer them for new targets; second, the DSBs they induce are primarily repaired through error-prone non-homologous end joining (NHEJ), which can lead to unintended mutations (19).

Zinc finger nucleases (ZFNs), TALENs and mega nucleases pioneered genome editing but have key limitations. ZFNs need complex protein design and often cause off-target effects (20). TALENs are easier to customize than ZFNs but are hindered by repetitive domain assembly and reduced efficiency in methylated regions (21). Meganucleases offer high specificity but are hard to retarget due to rigid recognition sites (22). To overcome this, CRISPR/Cas9, adapted from bacterial immunity, uses an RNA guide for precise and efficient genome editing. Its simplicity, low cost and versatility make it the most widely used gene-editing tool (23).

CRISPR-Cas 9 tool

CRISPR/Cas9 has emerged as a fast-evolving gene-editing tool, widely used in diverse species ranging from model organisms to key agricultural plants. Originally discovered in *Streptococcus pyogenes*, CRISPR short for Clustered Regularly Interspaced Short Palindromic Repeats and its associated protein *Cas9* (CRISPR-associated protein 9) have emerged as one of the most versatile genome editing tools (24). The CRISPR/Cas9 system introduces targeted DSBs at specific genomic sites, which are subsequently repaired by either homology-directed repair (HDR) or non-homologous end joining (NHEJ), enabling precise genetic modifications.

CRISPR/Cas systems have great potential for genome editing, managing off-target effects remains a significant challenge (25). Cas9 can cleave Non-specific sites in the genome, leading to off-target effects, primarily because it can tolerate up to three mismatches with the guide RNA (sgRNA) (26). Table 1 summarizes the strategies for validating genome edited bananas.

CRISPR/Cas 9 mechanism and structure: The CRISPR-Cas9 system is an RNA-guided adaptive immune mechanism found in many bacteria and archaea. It provides protection against bacteriophages and plasmids through a CRISPR locus composed of clustered repeat-spacer arrays along with CRISPR -associated (cas) genes (31). The CRISPR gene consists of identical repeats separated by spacers, forming a repeat-spacer array. The Cas1-Cas2 complex recognizes and cuts foreign DNA from bacteriophages or plasmids and inserts a new spacer upstream of a short 2-6 nucleotide PAM sequence. This foreign DNA gives the memory of its own genome, which will prevent the bacteria from phage infection in the future.

Table 1. Strategies for validating genome-edited bananas

Strategy	characteristics	Purpose	Reference
Whole-Genome Sequencing (WGS)	Sequencing the entire genome to detect both on-target and off-target mutations	Comprehensive validation of genome edits	(27)
In Silico Prediction Tools	Computational tools (e.g., CRISPOR, Cas-OFFinder, CCTop) predict potential off-target sites	Design and risk assessment of sgRNAs	(28)
PCR and Sanger Sequencing	Amplification and sequencing of the edited region to confirm edits	Verification of on-target mutations	(30)
T7 Endonuclease I (T7EI) Assay	Detects mismatches in re-annealed DNA indicating indels	Rapid screening of editing events	(29)

The Protospacer Adjacent Motif (PAM) is a short sequence (typically 2-6 bp) located immediately downstream of the target DNA in foreign genomes. It is essential for Cas9 recognition and cleavage, ensuring that only non-self DNA is targeted. The absence of PAM sequences in the CRISPR locus prevents self-targeting by the Cas9 protein. Fig. 1 explains the CRISPR/Cas9 immune system in bacteria.

DNA-free genome editing using CRISPR/Cas9 ribonucleoproteins (RNPs) overcomes the regulatory concerns associated with GMOs. This approach introduces pre-assembled Cas9 protein and guides RNA complexes directly into plant cells. Avoiding the use of foreign DNA lowers the chances of off-target effects and insertional mutations (32). Endogenous proteases and nucleases rapidly break down ribonucleoproteins (RNPs), which shortens the exposure time to genome-editing components and increases safety. Since RNPs do not introduce foreign DNA, they simplify the regulatory process and they may not fall under the stringent regulations applied to traditional GMOs (33). Using DNA-free genome editing methods like CRISPR/Cas9 RNP delivery supports the improvement of crops by easing public and regulatory concerns about GMOs (34).

Transcription of this new foreign DNA integrated repeat-spacer array will yield CRISPR RNAs (crRNAs). Now, the crRNA has a transcript of the foreign DNA (spacer) in the 5′ end and CRISPR repeats in the 3′ end. The tracrRNA, a short non-coding RNA complementary to the crRNA repeat sequence, base-pairs with crRNA to form an RNA-RNA hybrid. Cas9 binds this hybrid, forming the guide RNA (gRNA) complex that directs DNA targeting.

Cas9 is a large endonuclease encoded by a single gene and comprises seven functional domains (Fig. 2) these include the PAM-interacting (PI) domain, which recognizes the PAM sequence; the HNH and RuvC nuclease domains, responsible for cleaving the complementary and non-complementary DNA strands. Whereas the RECI, REC II, REC III, Bridge Helix domains and I which help stabilize the guide RNA within the protein (35).

Once the *Cas9-gRNA* complex identifies a PAM sequence in the targeted DNA, the guide RNA hybridizes to the complementary strand adjacent to the PAM. The PI domain ensures PAM recognition, while the HNH and RuvC domains cleave both the strands of the DNA approximately 3-4 nucleotides upstream of the PAM, creating a DSB (Fig. 3). This targeted cleavage allows for precise genome editing in engineered systems and provides adaptive immunity in bacteria by degrading foreign DNA. Table 2 summarizes the advancement in genome editing in horticultural crops.

Biotic stress management in banana

Bacterial disease management

A modified form of the CRISPR tool, CRISPRa increases gene

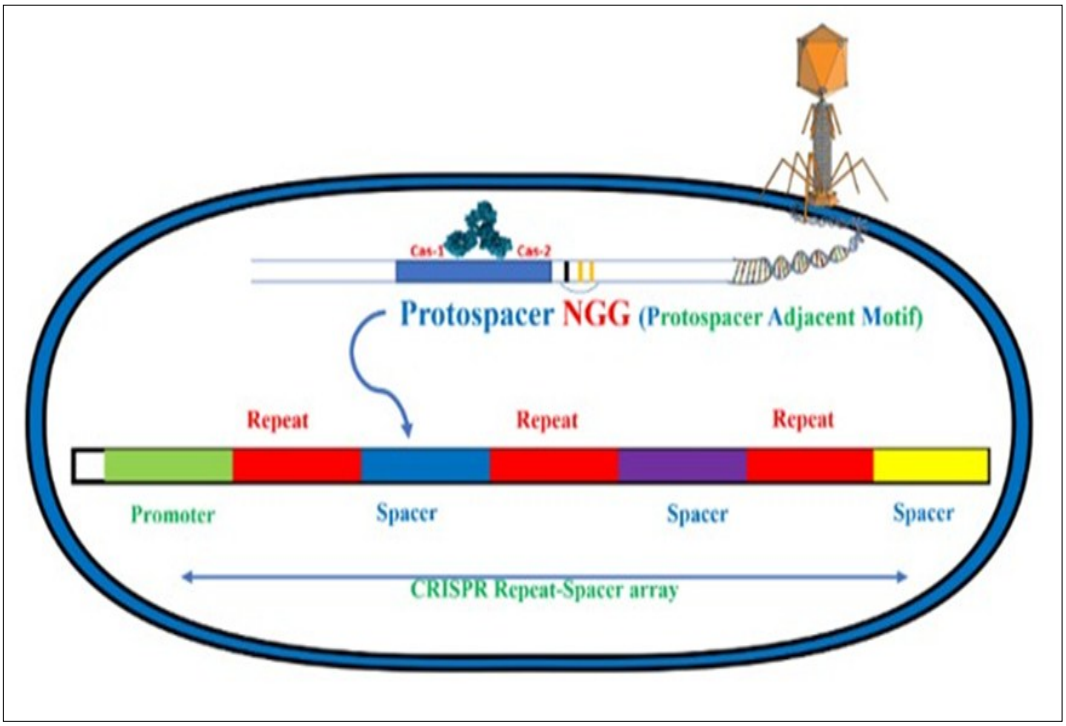

Fig. 1. Viral protospacer insertion (created in Biorender.com). The CRISPR/Cas, defence mechanism in bacteria, where fragments of viral DNA (protospacers) are stored in the CRISPR array as spacers. During subsequent infections, Cas proteins use these spacers to identify and cleave the corresponding viral DNA, with the help of the protospacer adjacent motif (PAM).

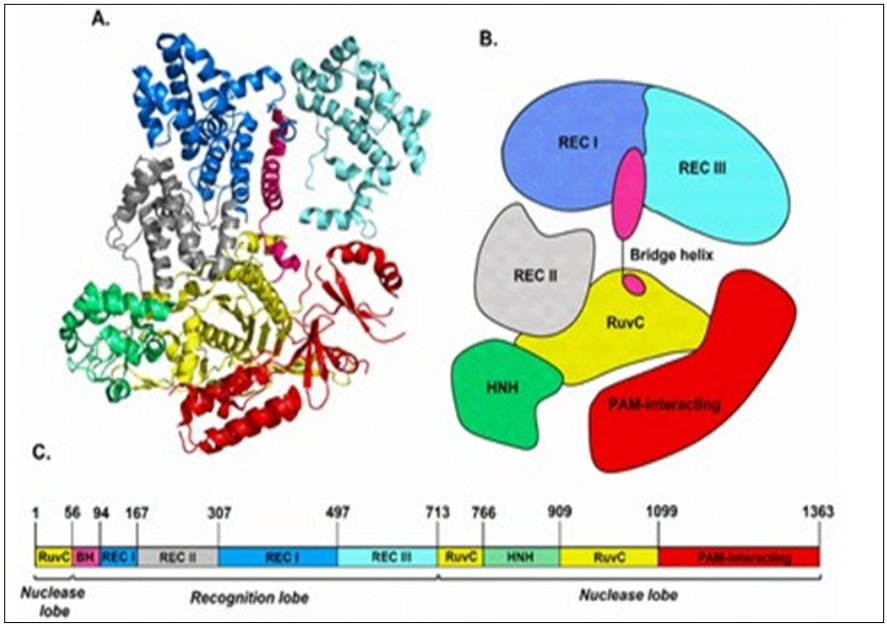

Fig. 2. Cas9 endonuclease structure (35). The *Streptococcus pyogenes* Cas9 protein consists of seven distinct domains: Rec I, Rec II, REC III, Bridge Helix, RuvC, HNH and the PAM-interacting domain.

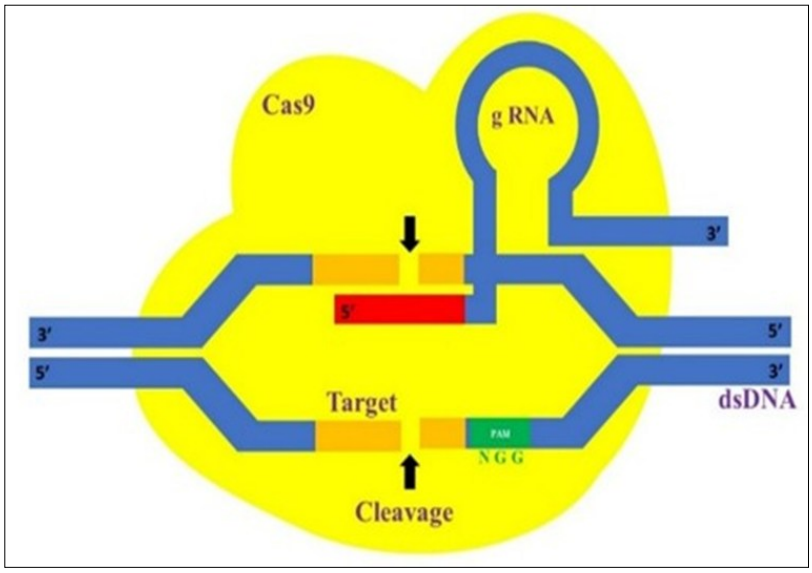

Fig. 3. Double strand break by Cas9 (created in Biorender.com). The Cas9 protein, guided by a gRNA (guide RNA), binds to a specific DNA sequence next to a PAM (NGG) site. Cas9 induces a double-stranded break (cleavage) at the target site, enabling precise genome editing.

expression by using transcriptional activators with deactivated Cas9 protein lacking endonuclease function (dCas9-dead Cas9). It is produced by altering the Cas9 protein's nuclease domain, which results in a lack of endonuclease function but maintains the ability to bind certain DNA sequences. Once the activation domain and dCas9 are combined, the insert gene can be transcriptionally active efficiently and accurately without producing any endogenous gene mutations (61).

Comparative transcriptomic analysis between Banana Xanthomonas Wilt (BXW)-resistant *Musa balbisiana* and the susceptible cultivar 'Pisang Awak' reveal several defense-related genes associated with resistance to BXW. These include antimicrobial proteins like Vicilin, Leucine-Rich Repeat (LRR) proteins, Wall-Associated Kinases (Wak2 and Wak5), Pathogenesis-Related (PR) proteins and disease resistance (R) genes such as the Musa RPM1 gene. Activation of these genes using CRISPRa/dCas9 technology conferred resistance to the otherwise susceptible 'Pisang Awak' cultivar (6).

Certain endogenous plant genes, termed susceptibility (S) genes, can facilitate pathogen colonization and symptom development during infection (6). One such gene, Downy mildew resistance 6 (DMR6) is atypically upregulated during bacterial infection and has been linked to increased susceptibility (62). DMR6 and its paralogs have been found to provide broad-spectrum resistance to bacterial infections. Therefore, editing MusaDMR6 orthologs offers an improved approach to manage BXW. Based on qRT-PCR investigation of in vitro plantlets, which revealed a 60-fold increase in gene expression upon *Xanthomonas campestris pv. musacearum* (Xcm) infection, the gene Ma04_p20880.1, one of the orthologs of MusaDMR6, was chosen to improve disease resistance in bananas. A plasmid construct, pMDC32-Cas9-MusaDMR6, containing a plant-codon-optimized Cas9 gene driven by a 2XCaMV35S promoter, two gRNAs targeting MusaDMR6 under OsU6 promoter separately and a plant selectable-marker hpt gene controlled by the CaMV35S promoter was made and inserted via Agrobacterium-mediated transformation into a susceptible cultivar Sukali Ndiizi. This transformation gave the mutation frequency of 100 % by deletion, insertion, or

Table 2. Advancement in genome editing in horticultural crops

Crop	Genome editing	Transmission method	Target gene	Trait associated with the gene	References
Apple cv. Golden delicious	CRISPR/Cas9 RNPs	Protoplasts	DspA/E interacting proteins of malus (DIPM 1-4)	Fire blight resistance	(36)
Apple cv. JM2	CRISPR/cas9	Agrobacterium	*Malus domestica* phytoene desaturase (MdPDS)	Albino devoid green tissue	(37)
Sweet potato	CRISPR-cas13	Agrobacterium	Sweet potato chlorotic stunt virus (SPCSV-RNase13)	Virus resistance	(38)
Straw berry	CRISPR/cas9	Agrobacterium	Tryptophan aminotranferase1 (TAA 1), *Fragaria vesca* auxin response factor (FveARF8)	Auxin biosynthesis response	(39)
Grapes cv.Thompson seedless	CRISPR/cas9	Agrobacterium	*Vitis vinifera* transcription factor (VvWRKY52)	Botrytis cinerea resistance	(40)
Grapes cv.Chardonnay	CRISPR/cas9	Agrobacterium	L-idonate dehydrogenase gene (LdnDH)	Biosynthesis of tartaric acid	(41)
Cassava	CRISPR/cas9	Agrobacterium	Cytochrome P450 genes Cytochrome P450 valine monooxygenase1 and valine monooxygenase 2 (CYP79D1) and (CYP79D2)	Cyanogen free cassava	(42)
Citrus	CRISPR/cas9	Agrobacterium	*Citus sinensis* lateral organ boundaries 1 (CsLOB1)	X. citri subsp. citri	(43)
Cassava	CRISPR/cas9	Agrobacterium	*Manihot esculenta* phytoene desaturase (MePDS)	-	(44)
Petunia	CRISPR/cas9	PEG-mediated protoplast transfection	Petunia nitate reductase (PhNR)	Deficiency in nitrate assimilation	(45)
Petunia	CRISPR/cas9	Agrobacterium	Petunia phytoene desaturase (PhPDS)	Albino type	(46)
Straw berry	CRISPR/cas9	Agrobacterium	Calmodulin-binding transcription activator (CAM)	Plant architechture	(47)
Straw berry	CRISPR/cas9	Agrobacterium	Sepallata 3 (SEP 3)	Flowering and fruit development	(48)
Apple	CRISPR/cas9	Agrobacterium	Phytoene desaturase (PDS)	Albino type	(37)
Kiwi	CRISPR/cas9	Agrobacterium	Centroradialis (CEN,CEN4)	Plant stature, early flowering	(49)
Chrysanthemum	CRISPR/cas9	Agrobacterium	*Chiridius poppei* yellowish-green fluorescent protein (CpYGFD)	Fluroscence protein disruption	(50)
Sweet orange	CRISPR/cas9	Agrobacterium	Downy mildew resistance 6 (DMR6)	Huanglongbing resistance	(51)
Sugarcane	CRISPR/cas9	Agrobacterium biolistics	Caffeic acid o-methyl transferase (SoCOMT)	Reduction in lignin content	(52)
Kiwi	CRISPR/cas9	Agrobacterium	Phytoene desaturase (PDS)	Albino type	(53)
Pear	CRISPR/cas9	Agrobacterium	Terminal flower 1 (TFL1)	Early flowering	(54)
Sweet orange	CRISPR/cas9	Agrobacterium	*Citus sinensis* transcription factor (CsWRKY22)	Canker disease resistance	(55)
Sweet orange	CRISPR/cas9	Agrobacterium	*Citrus sinensis* lateral organ boundaries (CsLOB1)	Canker disease resistance	(56)
Potato cv.desiree	TALENS	Protoplasts	*Solanum tuberosum* Acetolactate synthase 1 (StALS1)	Herbicide resistance	(57)
Potato cv.ranger russet	TALENS	Protoplasts	*Solanum tuberosum* Vacuolar invertase (StVLnv)	Cold induced sweetening	(58)
Cucumber	CRISPR/cas9	Agrobacterium	Eukaryotic translation initiation factor 4 E (eiF4E)	Resistance to cucumber vein yellowing virus (CVYV), papaya ring spot virus-type w (PRSV-W)	(59)
Tomato	CRISPR/cas9	Agrobacterium	Intergenic region coat protein-replication associated protein (IRCP-ReP)	Resistance to tomato yellow leaf curl virus (TYLCV)	(60)

substitution and partial to complete resistance against *Xanthomonas campestris* pv. *musacearum* Xcm (63).

Fungal disease management

Gabriella Kovács and colleagues were the first to demonstrate that resistance to *Mycosphaerella fijiensis*, the causal agent of Black Leaf Streak Disease in bananas, can be enhanced by overexpressing plant chitinase an enzyme that degrades fungal cell wall components (64). The binary vectors pBI333-EN4-RCC2 and pBI333-EN4-RCG3 contain the CaMV35S-driven hygromycin phosphotransferase (hpt) gene along with either the rcc2 or rcg3 rice chitinase gene, both regulated by a modified CaMV35S promoter (65). To provide resistance against Banana Black Leaf Streak Disease, which is caused by *M. fijiensis*. The two vectors were introduced into *Agrobacterium tumefaciens* strain EHA105 and subsequently used to transform embryogenic cell suspensions (ECS) of the dessert banana cultivar 'Gros Michel' (66).

Magainin, an antimicrobial peptide originally isolated from frogs, exhibits broad-spectrum activity by causing membrane depolarization, disrupting bacterial respiration and triggering autolytic enzymes. Therefore, the *Agrobacterium*-mediated transformation method is utilized to inhibit *Fusarium oxysporum* f.sp. *cubense*, which causes Panama wilt, in banana cultivar Rasthali by transforming the gene MSI-99, a synthetic analog of magainin (67). However, the use of a single anti-fungal gene for disease resistance may result in reduced resistance by adaptations of the fungi and natural selection, the need for the use of multiple genes for providing disease resistance became the point of interest for enhanced protection.

In another study, the authors introduced two antifungal genes the endochitinase gene ThEn-42 from *Trichoderma harzianum* and the grape stilbene synthase gene (StSy) into the banana cultivar Grand Nain using particle bombardment (68). This resulted in tolerance to Sigatoka disease under the control of the 35S promoter and the PR-10 inducible promoter. Because of its broad range of antifungal activity, these genes that exhibit tolerance to Sigatoka disease also exhibit tolerance to *Botrytis cinerea*.

Nematode management

A study demonstrated that Agrobacterium-mediated transformation of the rice cystatin gene OcIΔD86 provided limited resistance to the plant-parasitic nematode *Radopholus similis*, mainly due to low expression levels of the transgene (69). In addition, a higher level of cystatin expression than achieved may be of value and they expect additive resistance will be needed to achieve an effective and durable resistance. The transgenic plantain cultivar 'Gonja Manjaya,' which either alone or in combination expressed an anti-root invasion peptide and an anti-feedant cysteine proteinase inhibitor from maize. It subsequently showed strong resistance to *Radopholus similis* and *Helicotylenchus multicinctus* under both greenhouse and field conditions (5).

Control of viral diseases through genome editing (CRISPR-Cas 9)

Viral diseases are a major concern in banana cultivation because they affect the growth and quality of fruits. Farmers to cultivate fresh areas frequently use young suckers from former plantations. This strategy has played a significant role in the global spread of a number of banana diseases and pests, especially viruses like Bunchy Top Virus (BBTV) and Banana Streak Virus (BSV) (70).

The CRISPR/Cas system is an emerging tool to control plant viruses. CRISPR/Cas9 is a diverse, cost-effective and efficient approach for generating viral resistance. Further, it can be used to maximize crop output, grain quality and nutritional content. In general, many plant viruses have DNA/RNA as genetic material. CRISPR/Cas 9 directly targets the DNA viruses by cleaving the genomes. For RNA viruses, newer CRISPR systems such as Cas13, which specifically target RNA, offer potential avenues for developing resistance.

Banana bunchy top disease: Banana Bunchy Top Disease (BBTD) is considered the most destructive disease affecting banana cultivation (71). It was first reported in Fiji in 1889, although its viral cause was only confirmed nearly a century later (72). Banana Bunchy Top Virus (BBTV), a circular, multi-component, single-stranded DNA virus, causes the disease. The banana aphid persistently transmits BBTV magge (*Pentalonia nigronervosa*). Although the virus cannot spread by sap, it can spread through infected plant suckers and other plant parts used to propagate bananas. The initial symptoms of infection are dark-green streaks on the leaf veins, midrib and petiole, which vary in length. Plants infected early in their growth do not produce fruits, resulting in total production loss, but plants infected at later stages may produce normal or malformed fruits (71, 73).

Transgenic hill banana plants expressing RNAi-BBTV rep showed resistance to BBTV infection. The transformed plants show no symptoms and have practically no replication of challenge BBTV. The detection of small interfering RNAs (siRNAs) derived from the intron-hairpin RNA (ihpRNA) transgene in BBTV-resistant transgenic plants confirms that RNA interference (RNAi) is the underlying mechanism of resistance. This RNAi-mediated resistance effectively controls BBTV infection in hill bananas (74, 75).

However, complete gene silence is not possible from RNAi interference; hence, CRISPR/Cas 9 is used for gene editing of target genes. The eIF4E and its paralogue, eIF (iso) 4E, are part of the eukaryotic translation initiation factor (eIF) gene family. Editing of eIF4E has been employed to confer resistance against several viruses, including rice tungro spherical virus, papaya ringspot virus-type W, cucumber vein yellowing virus, zucchini yellow mosaic virus and cassava brown streak virus, along with modifications involving eIF4G (59, 76). Although BBTV is a DNA virus, editing host susceptibility factors like eIF genes could potentially offer resistance, as similar mechanisms have been observed in other plant-virus interactions.

Banana Streak Disease (BSV): Banana Streak Disease (BSV) is considered one of the most widespread diseases affecting banana plantations worldwide. The first outbreak was reported in 1958 in the Nieky Valley of Ivory Coast, primarily affecting the triploid cultivar 'Gros Michel' (57). BSV is a group of viruses that belong to the Para Retrovirus family and are characterized as endogenous Para Retroviruses when they integrate into a host genome. Endogenous BSV (eBSV) refers to BSV that has been incorporated into the banana genome (5). The banana genome produced from *M. balbisiana* is

where the eBSV sequences are mostly integrated (77). Depending on the viral strain, host cultivar and environmental factors, BSV symptoms can range from a mild chlorotic flush to lethal necrosis (78).

Mechanical transmission of BSV is not possible. However, the virus is disseminated over long distances via vegetative propagation through infected suckers and micropropagated plantlets (79). Mealybugs transmit the virus in a semi-persistent manner, spreading it from infected banana plants to healthy ones (80). The infected fruits are smaller, distorted and have thinner peels.

Banana Bract Mosaic Disease (BBrMV): Banana Bract Mosaic Virus (BBrMV), a member of the genus Potyvirus, is a major viral pathogen of banana that severely affects yield and productivity, particularly in India and the Philippines (81). On bracts, pseudo stems, midribs, peduncles and even on fruits, the virus-infected plants have spindle-shaped violet stripes (82). The virus is transmitted in a non-persistent manner by several aphid species, including *Aphis nigronervosa*, *Rhopalosiphum maidis*, *Aphis gossypii* and *A. craccivora* (83, 84). A virus-resistant banana line was developed through genetic engineering by who designed intron-hairpin RNA (ihpRNA) constructs targeting essential BBrMV genes. The constructs were initially inserted into the binary vector pSTARLING; and later transferred to the vector PART27 for Agrobacterium-mediated plant transformation. The transformed plants exhibited resistance to BBrMV, demonstrating the effectiveness of RNAi in combating this virus.

Abiotic stress management in banana

Abiotic stress in banana

Abiotic stresses are caused by non-living environmental factors such as extreme temperatures, salinity, light intensity (including darkness and UV radiation), water availability (drought and flooding), nutrient imbalances and oxidative damage. Banana is vulnerable to a wide range of abiotic stresses such as high salinity, drought, high temperature, strong winds and water logging. These stresses may hinder plant growth and flower and fruit development leading to substantial loss in yield. Gene editing has shown significant potential in enhancing abiotic stress tolerance in crops, although research in this area remains relatively limited. To date, the application of CRISPR/Cas9 for improving tolerance to abiotic stresses has been demonstrated primarily in crops such as maize, wheat and other crops. Knocking out the 4-coumarate ligase (4CL) and Reveille 7 (REV7) genes in chickpea transgenic lines has led to enhanced drought tolerance. Moreover, the CRISPR-Cas9 system offers a way to develop non-transgenic chickpea plants with desirable traits, potentially increasing yield under abiotic stress conditions (91). CRISPR/Cas9 used to create *osera1* rice mutants, which showed enhanced root growth and greater sensitivity to ABA (92). These mutants responded better to drought through improved stomatal regulation, identifying *Oryza sativa* ERECTA 1 (*OsERA1)* as a key negative regulator of drought tolerance in rice. In the case of bananas, emerging studies have begun to explore the use of gene editing to address similar challenges. Table 3 summarizes recent gene-editing strategies employed to enhance abiotic stress tolerance in banana.

Overexpressing Aquaporin genes for abiotic stress tolerance

Aquaporins (AQPs) are channel proteins located in cell membranes that enable the efficient transport of water and small molecules across biological membranes; however, their precise function in responding to abiotic stresses has advanced in recent times (93). Investigated six rice varieties to assess aquaporin roles in root water transport under well-watered and drought conditions. Aquaporins contributed up to 85 % to root hydraulic conductivity and showed increased activity during drought. Their expression and root anatomy correlated with hydraulic traits, revealing additional genetic factors beyond known aquaporin genes.

Overexpression of Aquaporin genes in banana improved tolerances to multiple stresses (94). The Aquaporin genes such as (*Musa accuminata* plasma membrane intrinsic protein) *MaPIP1;1* and *MaPIP2;7* were transferred to the pCAMBIA1302 expression vector and transformed into banana (*Musa acuminata* cv. Mas) with *Agrobacterium*-mediated transformation to evaluate the effect of abiotic stress tolerance by Aquaporins (61). *MaPIP1;1* and *MaPIP2;7* are aquaporin genes in banana that help plants cope with drought and salt stress by regulating water transport and ion balance. *MaPIP1;1* expression increases under drought and salinity, enhancing water uptake and reducing membrane damage (89). *MaPIP2;7* is highly upregulated under salt stress and its overexpression improves salt tolerance by lowering Na^+ and K^+ levels and increasing the K^+/Na^+ ratio (95).

Drought stress

To evaluate drought tolerance, 100-day-old wild-type and transgenic banana lines overexpressing *MaPIP1;1* and *MaPIP2;7* were subjected to drought stress (61). Most of the mutant plants' leaves stayed green after 10 to 15 days without water, but the leaves of the wild type plants curled and turned chlorotic. Following a 10-day watering period, the transgenic

Table 3. Genome editing in banana for different abiotic stress

Specific protein/gene	Abiotic stress tolerance	Trait associated with gene	Reference
MaCCS gene	Heat, cold, drought and light	Delivers copper to its target Cu/ZnSODs. Function is not identified yet	(85)
Musa acuminate CAMTA1	Drought	No study has conducted on MuCAMTA1, to identify their role in drought stress with respect to banana plants	(86)
MaSWEET genes	Drought, cold, salt	Encourages early sugar transfer in order to increase banana stress tolerance and fruit quality	(87)
ATPase and heat shock proteins	Drought	It involves in growth and development and stress responses	(88)
MaZIP genes	Drought, cold, salt	Involves in stress signalling, but their function is not identified	(89)
M. acuminata root hair defective 3 (MaRHD3)	Drought	ROS scavenging increased the density of root hair and lateral root branching	(90)

plants demonstrated enhanced growth and vitality compared to wild-type plants during a subsequent 15-day drought. Transgenic plants under drought stress possessed higher levels of proline, chlorophyll, soluble sugar and abscisic acid (ABA) plays a role in reducing membrane damage and maintaining the osmotic balance of plant cells under drought conditions. Additionally, these lines had lower levels of abscisic acid (ABA), malondialdehyde (MDA a marker of lipid peroxidation) and ion leakage (IL), indicating reduced oxidative damage and enhanced membrane stability under drought conditions.

Salt stress

The overexpression of aquaporin genes has also been associated with improved salt stress tolerance in banana. After 13 days of salt exposure followed by a 10-day recovery period, transgenic lines exhibited a greater proportion of green leaves and significantly, less root damage compared to wild-type plants, indicating enhanced salt resilience. Salt stress activates *MaPIP2;7*, which reduces cellular Na^+ and K^+ levels and increases the K^+/Na^+ ratio. This shift in ion balance helps maintain cellular homeostasis. Consequently, *MaPIP2;7* enhances the plant's salt tolerance (96).

Cold stress

Under cold stress conditions, wild-type banana plants exhibited significant leaf yellowing after 5 days at 8 °C, followed by an 11-day recovery period. In contrast, transgenic lines overexpressing aquaporin genes maintained better physiological condition and showed less visible damage (61).

Shelf-life extension in banana

Transcriptional regulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) genes influences the production of ethylene in climacteric fruits. ACC is converted to ethylene by ACO during the ripening phase. More significant than any other ACO (about 18 ACOs have been identified from *Musa acuminate*), the MA-ACO1 gene is involved in the synthesis of ethylene by expression analysis (89). To reduce ethylene biosynthesis, *MaACO1* disrupted mutants were developed using the CRISPR/Cas9 system with sgRNA (single guide RNA) sequence of 5′-CCTCATGGATGAAGTGGAGAAGG-3′ that targets the second exon of MaACO1 (97). On evaluation, all six mutant (MaACO1 disrupted) lines gave a lower yield compared to the wild types. The main challenge in gene editing for longer shelf life is that delaying ripening often reduces crop production. They chose two out of six mutant plants and tested them at room temperature, along with normal plants, to see how the mutation affected shelf life after harvest.

The wild-type fruits ripened 21 days after harvest, whereas ripening was significantly delayed in the MaACO1-disrupted lines. Yellow coloration and brown speckle formation appeared in the wild-type fruits within 21 days, but the mutant fruits remained green or slightly yellow without speckles even after 60 days. However, when both types were treated with ethephon, they ripened normally. Fig. 4 explains the difference between ripening behaviour in wild types and ethephon treated (97).

Constraints in genome-editing in banana

CRISPR/Cas9-based technologies primarily use Agrobacterium to deliver genes, inserting random foreign DNA into plant genomes and creating genetically modified (GM) crops. Although this method is effective, it requires extensive tissue culture and regeneration. However, it is unsuitable for vegetative propagated crops, where removing transgenes through segregation is not possible (98). One of the major challenges in the genome editing of banana is targeting multiple alleles and gene copies at the same time. In bananas, knocking out or reducing the expression of a single gene often shows little or no visible effect on the plant because other similar gene copies compensate for it a phenomenon known as the dose effect. In bananas, a polyploidy crop, multiple gene copies (paralogs) often exist due to their triploid or tetraploid genome structure. As a result, the alteration or knockout of a single gene copy may not produce a noticeable phenotype, since other functional paralogs can compensate. This phenomenon is known as the gene dosage or paralog compensation effect (99). If CRISPR/Cas9 knocks out only one allele like MaACO1, other paralogous genes (e.g., MaACO2, MaACO3) can still produce enough ethylene for ripening. As a result, the trait remains unchanged due to paralog compensation (87). To obtain edited lines with multi-allelic mutations, guide RNAs (gRNAs) must target all gene copies and alleles simultaneously through careful design. Additionally, screening a large number of mutants is necessary to identify successful edits. In polyploidy crops like bananas, this

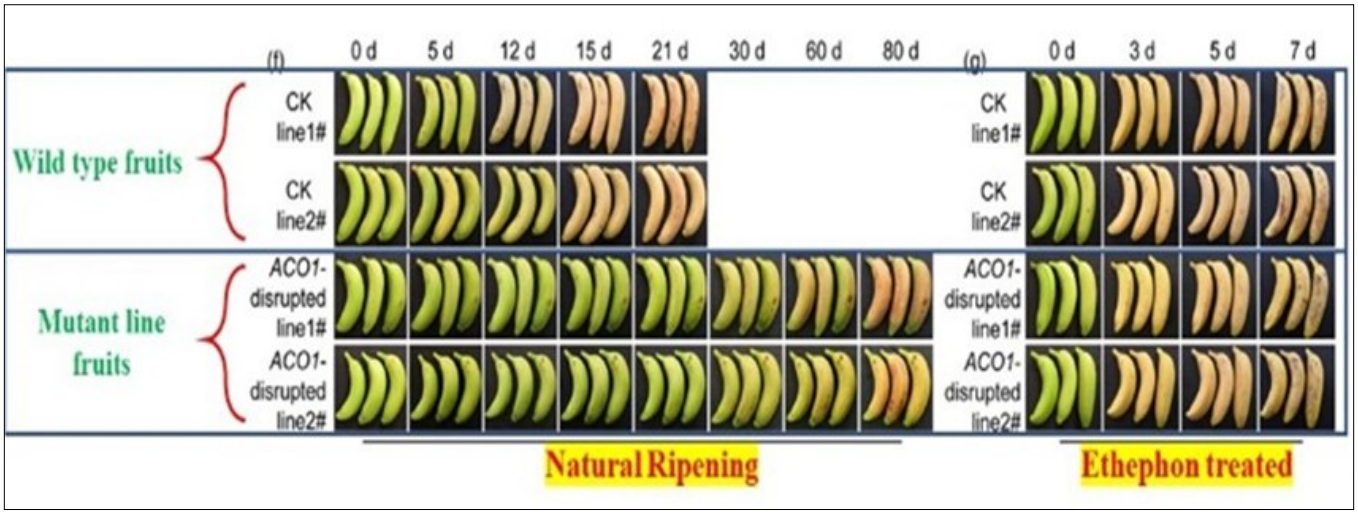

Fig. 4. The shelf-life study under 22 °C (97). The fruit ripening behaviour of two MaACO1-disrupted mutant lines and the wild type under natural conditions at 22 °C. It also assessed ripening in both the mutants and the wild type following ethephon treatment.

challenge can be effectively addressed through multiplexed genome editing, which employs multiple gRNAs to target several genes and their paralogs within a gene family (100).

Conclusion

Gene editing technologies offer a precise and efficient approach to developing cultivars with enhanced resistance to both biotic and abiotic stresses, improved fruit quality and extended shelf life. Although conventional breeding has played a crucial role in developing cultivars resistant to abiotic and biotic stresses, the process is often complex, time-consuming and labour-intensive. These challenges can be overcome with the application of advanced technologies such as RNA interference (RNAi) and CRISPR/Cas9-based systems, which offer greater efficiency and high target specificity for stress management in crops. Despite ongoing ethical and regulatory considerations, CRISPR/Cas9 has emerged as a highly promising tool in modern crop improvement strategies. In banana, a crop facing substantial biotic and abiotic pressures, gene editing holds great promise for the development of resilient and sustainable cultivars. Remarkably, this technology has been employed to address a wide range of agricultural challenges within just a few years of its introduction.

Future thrusts

The researchers should focus on DNA-free approaches like CRISPR/Cas9 RNP complexes, to avoid foreign gene insertion and to reduce the off-targets. To overcome the regulatory barriers in banana genome editing, non-transgenic delivery methods to be developed. There is a need to develop the protoplast regeneration, nano -particle delivery and viral vectors for accurate gene editing. These strategies are crucial for vegetative propagated crops, such as banana, where segregation of genes is not possible.

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Authors' contributions

VK contributed to the collection of articles and formulated the concept. MMI prepared the draft for the review. SD, SS and MBN compiled the references. AJ and SR corrected and revised the manuscript. All the authors read and approved the final manuscript.

Compliance with ethical standards

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