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Precision genetics tools for genetic improvement of banana

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Abstract

Banana is an important food security crop for millions of people in the tropics but it faces challenges from diseases and pests. Traditional breeding methods have limitations, prompting the exploration of precision genetic tools like genetic modification and genome editing. Extensive efforts using transgenic approaches have been made to develop improved banana varieties with resistance to banana Xanthomonas wilt, Fusarium wilt, and nematodes. However, these efforts should be extended for other pests, diseases, and abiotic stresses. The commercialization of transgenic crops still faces continuous challenges with regulatory and public acceptance. Genome editing, particularly CRISPR/Cas, offers precise modifications to the banana genome and has been successfully applied in the improvement of banana. Targeting specific genes can contribute to the development of improved banana varieties with enhanced resistance to various biotic and abiotic constraints. This review discusses recent advances in banana improvement achieved through genetic modification and genome editing.

1 | INTRODUCTION

Bananas, including plantain (*Musa* spp.), are crucial for food security. They are grown in more than 145 countries and islands in tropical and subtropical regions, occupying over 12 million ha of land globally and about 7.5 million ha in Africa. The most prominent producers are India, China, the Philippines, Ecuador, and Brazil (FAOSTAT, 2021). According to FAOSTAT's recent data, the annual production of banana, including cooking banana and plantain, is about 170 million tonnes globally, with Africa contributing about 55 million tonnes (FAOSTAT, 2021). Today, bananas are one of the world's most essential fruits and food crops, with over 100 billion bananas consumed every year, providing income and food security to more than 400 million smallholder farmers globally (L. Tripathi et al., 2019). In East African countries such as Burundi, Rwanda, and Uganda, banana offers 30%–60% of the daily per-person calorie intake, with the highest consumption in Uganda (J. Dale et al., 2017a). It is a tropical fruit and a staple food originating in Southeast Asia, mainly in the Philippines and Malaysia (Simmonds, 1962).

Banana cultivars are polyploids originating from *Musa accuminata* (AA genome) and/or *Musa balbisiana* (BB genome). The total number of cultivars of bananas, including plantains, has been estimated to be around 300–1000, belonging to several genomic groups such as AA, BB, AB, AAA, AAB, ABB, AAAA, AAAB, AABB, and ABBB (Ploetz et al., 2007).

Banana production faces numerous challenges, including biotic and abiotic stresses, declining soil fertility, limited genetic diversity in germplasm, and insufficient

Abbreviations: AChE, acetylcholinesterase; BBrMV, banana bract mosaic virus; BBTV, banana bunchy top virus; BSV, banana streak virus; BXW, banana Xanthomonas wilt; dCas9, dead Cas9; DMR6, downy mildew resistance 6; DNA, Deoxyribonucleic acid; eBSV, endogenous banana streak viruses; ECS, embryogenic cell suspension; EFR, elongation factor receptors related; Hrap, hypersensitivity response-assisting protein; IITA, International Institute of Tropical Agriculture; PDS, phytoene desaturase; Pflp, plant ferredoxin-like protein; PRR, pattern-recognition receptor; RNP, ribonucleoprotein; TR4, tropical race 4.

availability of clean planting material, especially among smallholder farmers. Moreover, climate change and unpredictable weather patterns exacerbate the decline in banana yields, particularly in regions with minimal or nonexistent irrigation. Given the critical role of bananas as a staple food crop in Africa, it is imperative to focus on genetic improvement strategies to enhance the crop's tolerance to biotic and abiotic stresses. One such approach is the utilization of precision genetic tools like genetic modification and genome editing to develop improved banana varieties with higher yields and resistance to diseases and pests. Currently, extensive efforts are underway to employ these techniques to enhance banana production through the development of disease-resistant and pest-resistant varieties.

This article provides a comprehensive overview of constraints and limitations for banana production as well as advancements in precision genetic tools, particularly genetic modification and genome editing, to develop high-yielding disease- and pest-resistant bananas. By looking into the current state of research and the promising future prospects, this article will shed light on the possibilities of precision genetic tools in improving banana cultivation and addressing the challenges faced by smallholder farmers.

Core Ideas

- Banana faces significant challenges from diseases and pests that reduce yield and economic viability.
- Precision genetics tools like CRISPR/Cas offer solutions for developing disease and pest-resistant crops.
- Recent advances in genome engineering have opened possibilities for enhancing banana productivity.
- Precision genetics requires addressing regulatory concerns to ensure successful commercialization.

2 | LIMITATIONS IN BANANA PRODUCTION

Banana suffers from biotic and abiotic production constraints. The crop is susceptible to several plant pathogens like fungal, viral, and bacterial, and pests like nematodes and weevils, causing significant damage to the crop and reducing yields. This susceptibility is exacerbated due to a monoclonal cropping system, low genetic diversity, and clonal propagation using suckers isolated from parent plants, which increase the risk of disease transmission and hinder germplasm movements.

One of the primary biotic constraints in banana production is Fusarium wilt, a fungal disease also known as Panama disease, caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). It affects the vascular system of the plant, causing wilting and, ultimately, death. Foc is a soilborne fungus that multiplies asexually, producing microconidia, macroconidia, and chlamydospores. Foc persists in the soil for decades, making disease management challenging (Hennessy et al., 2005). According to Cook et al. (2015), Fusarium wilt will spread over time and cause banana on-farm losses exceeding $138 million per year despite a slow rate of spread and using all the preventive measures in the Northern Territory of Australia. There are several strains of Panama disease, with some being more virulent than others (Ploetz, 2015). Foc race 1 is widely distributed globally and responsible for the Gross Michael epidemic, race 2 affects cooking bananas, and race 4 splits into subtropical race 4 and tropical race 4 (TR4; Ploetz, 2015). For more than 20 years, TR4 has been contained in the Northern Territory of Australia and parts of Southeast Asia; however, since 2010, the disease has spread to additional countries in Southeast and South Asia, the Middle East, South America, and Africa (European and Mediterranean Plant Protection Organization, 2023). In 2013, TR4 was reported for the first time in Africa, in Mozambique. In 2019, TR4 was found in Colombia, which is the biggest banana exporter in the world. Recently, it has been reported in Israel, Jordon, Turkey, Mayotte, Peru, and Venezuela. The emergence of TR4 is of great concern as it is the deadliest fungal strain. The spread of TR4 in Africa is particularly alarming, considering that the continent is the world's second-largest banana producer (Viljoen et al., 2020; van Westerhoven et al., 2022). In Mozambique, TR4 has spread from commercial farms into smallholder production systems. Currently, no effective control measures are available for the prevention and management of Foc (Ploetz, 2015). Chemical control or biocontrol methods are ineffective or partially effective. Fusarium spores, mainly chlamydospores, remain viable in the soil for several years, making infested ground unsuitable for cultivating disease-prone banana varieties (Ploetz, 2015). The only option for controlling the disease is using Foc-resistant banana varieties generated by either banana breeding or genetic engineering (J. Dale et al., 2017a).

Black Sigatoka caused by *Mycosphaerella fijiensis* Morelet is another fungal disease affecting banana production. It causes black spots on the leaves, leading to premature defoliation, reduced yields, and poor fruit quality (Arango Isaza et al., 2016; Ploetz, 2001). Black Sigatoka was first identified in the Sigatoka Valley of Fiji in 1963, where it got the name black Sigatoka. It was first seen in 1972 in Honduras and spread to central Mexico, Bolivia and Brazil, the Caribbean islands, and Florida. In Africa, the disease was first found in Zambia in 1973 and has since spread throughout the sub-Saharan banana-growing region. Black Sigatoka is now gradually replaced by yellow Sigatoka as the predominant leaf spot disease of bananas (Ploetz & Mourichon, 1999).

Several viral diseases are also destroying banana plantations. *Banana bunchy top virus* (BBTV), *banana streak viruses* (BSVs), and *banana bract mosaic virus* (BBrMV) are the most prominent viral pathogens affecting bananas. BBTV (genus *Babuvirus*, family *Nanoviridae*) is the deadliest banana virus globally, causing up to 100% yield reduction in banana-growing regions (Kumar et al., 2015). It is most prevalent in Africa and the Asia-Pacific region. In Africa, BBTV is considered invasive, and over the last decade, BBTV has spread to at least eight countries (Benin, Cameroon, Mozambique, Nigeria, South Africa, Tanzania, Uganda, and Zambia) (Ocimati et al., 2021; Shimwela et al., 2022; Alliance for Control Banana Bunchy Top Disease in Africa [www.bbtvalliance.org], 2021). It is spread by the banana aphid (*Pentalonia nigronervosa)* and affects the entire plant, including the leaves, stem, and fruit. Infected plants produce smaller, distorted fruits and can eventually die (J. L. Dale et al., 1987). This disease is challenging to control and continuously spreading in Africa, where smallholder farmers primarily produce bananas. The disease can be managed by controlling insect vectors, using disease-free clean planting materials, and removal of the infected plants. Host plant resistance is the most appropriate control measure. There is no identified resistance to BBTV in the *Musa* germplasm. All types of banana cultivars grown are susceptible to BBTV.

BSV (genus *Badnavirus*, family *Caulimoviridae*) is a pararetrovirus causing chlorotic and necrotic streaks on leaves, pseudostem, and smaller, malformed fruit (Harper & Hull, 1998). It was first identified in banana-growing regions in West Africa (Fargette et al., 2006; Harper et al., 1999). Like other badnaviruses, BSV integrates into the B genome of banana, particularly plantain. BSV is a complex of different viruses classified as endogenous when integrated into a host genome. The BSV integrated into the banana host genome is known as endogenous banana streak viruses (eBSV). The virus exists in two forms, the integrated and episomal versions. Under unfavorable conditions such as drought, the integrated version activates episomal, causing disease symptoms. Due to this reason, BSV is considered a major constraint in banana breeding programs, restricting the use of the diploid progenitor *Musa balbisiana* or its derivates carrying a B genome as parents for introgression of desirable agronomic traits (Duray et al., 2016). It also prevents the germplasm movement of banana varieties with the B genome due to this potential activation of eBSV into the episomal infectious form of the virus. It is a severe viral disease spread by mealybugs and banana suckers. BBrMV spreads through aphids, affecting the banana fruit's floral bracts or protective coverings. Infected plants produce smaller, malformed fruit, which can reduce yields. It is mainly prevalent in India and the Philippines (Kumar et al., 2011).

Bacterial diseases, such as banana Xanthomonas wilt (BXW), caused by *Xanthomonas campestris* pv. *musacearum* is a threat to banana production in Africa. The bacterial infection leads to wilting, yellowing of the leaves, premature ripening of fruits, and finally, death of the plant. Plants are infected by insects carrying bacterial inoculum through the inflorescence or soilborne bacterial inoculum through the plant's roots and infected planting material (Biruma et al., 2007; L. Tripathi et al., 2009). Management practices are based on reducing inoculum pressure, like removing male buds after fruiting and using sterile cutting tools (Biruma et al., 2007). The banana yield losses are very severe in the Democratic Republic of Congo (83%), Uganda (71%), and in other East African countries like Burundi, Kenya, Rwanda, and Tanzania, it is about 39%–51% (Ainembabazi et al., 2015). None of the cultivated banana varieties are found to be resistant except for the wild-type progenitor *Musa balbisiansa* (Nakato et al., 2019). Losses due to bacterial wilt disease are about $2–$8 billion in a decade (Abele & Pillay, 2007).

Plant-parasitic nematodes and weevils are a significant global threat to banana production, causing annual yield loss of up to 40%–50% (Gold et al., 2001). Various nematodes like *Radopholus similis*, *Pratylenchus goodeyi*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus*, and *Meloidogyne* spp. are prevalent either alone or in combination in banana fields (Coyne et al., 2013). Commercial growers can apply nematicides to control nematodes, but this is environmentally unsafe and not affordable for smallholder farmers. Similar to nematodes, banana weevils (*Cosmopolites sordidus*) are the most challenging insect pest globally, causing severe damage to roots and pseudostem (Twesigye et al., 2018).

In addition to biotic constraints, abiotic factors also limit banana production. Rapidly changing climate conditions, including drought, changing temperatures, unpredictable rain patterns, and poor soil fertility, are negatively impacting crop productivity, and agronomic traits (Dhanker & Foyer, 2018; Elad & Pertot, 2014). Bananas require warm and humid conditions to grow, ranging from 25˚C to 32˚C. Extreme heat, cold, drought, excessive rainfall, soil moisture deficit, salinity, and strong winds can reduce banana production (Ravi & Vaganan, 2016).

To overcome these biotic and abiotic constraints, efforts must focus on developing disease-resistant varieties, implementing integrated pest management strategies, improving soil health, and adopting climate-smart cultivation practices. Only through comprehensive approaches, the limitations in banana production can be effectively addressed, ensuring sustainable and resilient banana farming systems.

3 | ADVANCES IN THE IMPROVEMENT OF BANANA USING PRECISION GENETICS TOOLS

Several intensive efforts have been made to develop disease- and pest-resistant banana to increase production through

genetic engineering approaches, including transgenic and genome-editing technologies.

3.1 | Genetic transformation of banana

A robust genetic transformation system plays a pivotal role in enhancing bananas' characteristics and disease resistance. Embryogenic cell suspensions (ECSs) are the most preferred and desired explants to generate transgenic and genome-edited bananas. Using ECS, researchers can generate 30–50 independent events in an experiment, which is very efficient (J. N. Tripathi, Oduor, et al., 2015). To generate ECS of banana, two types of explants are commonly used, immature male flowers or multiple meristematic tissues, also known as scalps. These explants are cultured on the callus induction medium supplemented with plant growth regulators to stimulate cell division and differentiation (Escalant et al., 1994; Novak et al., 1989; J. N. Tripathi et al., 2012; J. N. Tripathi, Oduor, et al., 2015). After 16–20 weeks, small clusters of friable calli develop on the surface of the callus induction medium. These calli are then transferred to a liquid callus induction medium and incubated in dark on the shaker at 95 rpm for several months to generate ECS. The generated ECS has enormous potential for rapid proliferation, reaching significant quantities within 2 weeks. The rapidly multiplying ECS can be used for genetic transformation to generate transgenic or genome-edited events. The utilization of ECS as explants for genetic transformation offers several advantages, including the ability to produce large numbers of events, accelerate the development of resistant banana varieties, and ensure uniformity and consistency in the transformed events.

The International Institute of Tropical Agriculture (IITA), Kenya, has established a state-of-the-art laboratory system for developing and maintaining ECS of various banana and plantain cultivars. Notably, successful generation of ECS has been achieved for Agbagba, Cavendish, Gonja Manjaya, Gros Michel, Orishele, Sukali Ndiizi, and Zebrina. These ECSs have been evaluated for regeneration, transformation efficiency, and genome editing efficiency, demonstrating their potential for trait improvement (Ntui et al., 2020; J. N. Tripathi, Oduor, et al., 2015).

Agrobacterium-mediated transformation using ECS has proven successful in introducing desirable traits such as disease resistance in banana (L. Tripathi, Tripathi, et al., 2019). This process involves using a highly virulent strain of the bacterium *Agrobacterium tumefaciens*, which transfers a piece of its deoxyribonucleic acid (DNA) called T-DNA into the genome of banana cell. The T-DNA can be engineered to carry the gene(s) of interest, such as those conferring resistance to a particular disease. The transformed cells are subsequently cultured on a nutrient-rich medium containing plant growth regulators, fostering the regeneration of plantlets from the transformed cells. These plantlets are then transferred to soil and grown to maturity (Ganapathi et al., 2001; Khanna et al., 2004; J. N. Tripathi, Oduor, et al., 2015). By utilizing *Agrobacterium*-mediated techniques, ECS of banana has demonstrated successful transformation for various traits, including resistance against bacterial, fungal, and viral diseases such as BXW, Fusarium wilt, BBTV, BSV, and nematodes that pose severe threats to banana production (L. Tripathi, Tripathi, et al., 2019).

The genetic transformation of bananas using ECS has paved the way for significant advancements in banana improvement by enhancing disease resistance and improving agronomic traits.

3.2 | Developing disease- and pest-resistant banana through a transgenic approach

Fusarium wilt poses a significant threat to banana cultivation worldwide. Researchers have made progress in developing disease-resistant bananas through transgenic approaches (Table 1). Several transgenes have been investigated, demonstrating resistance against fungal pathogens. Transgenic banana engineered with rice *thaumatin-like protein* or *PR-5* gene has shown significant resistance against Foc race 1 (Mahdavi et al., 2012). Similarly, the overexpression of the *anti-apoptosis* gene in transgenic bananas showed enhanced resistance against Foc race 1 (Magambo et al., 2016; Paul et al., 2011). Additionally, RNAi silencing of targeted genes of Foc race 1 showed significant resistance in transformed events (Ghag et al., 2014). Transgenic Cavendish bananas overexpressing the *RGA2* or *Ced9* gene exhibited enhanced resistance against Foc TR4 under confined field trials, offering a potential solution to combat this devastating disease (J. Dale et al., 2017b). In this case, the resistance gene from a wild banana that is immune to TR4 was inserted into Cavendish. Recently, these TR4-resistance transgenic Cavendish bananas, known as QCAV-4, have been sent to regulators for approval to be released for commercial use in Australia (The Guardian, 2023).

Transgenic banana Gros Michel overexpressing the rice *chitinase* gene displayed resistance to black Sigatoka disease (Kovács et al., 2013). Similarly, stacking the *Trichoderma harzianum endochitinase* gene with grape *stilbene synthase* in the transgenic banana conferred complete resistance to fungal attacks (Vishnevetsky et al., 2011). These transgenic events were further tested through 4-year field trials, and several banana events showed tolerance to black Sigatoka disease.

In the quest to develop resistance against BBTV, several researchers have applied RNAi technology to silence the essential viral genes (Elayabalan et al., 2013; Shekhawat et al., 2012). Although RNAi does not entirely silence the targeted genes, it seems promising in reducing BBTV infection. At IITA and in collaboration with the Queensland University of Technology, efforts have been made to

TABLE 1 Scientific advances in the precision genetic of banana using transgenic and CRISPR/Cas-based genome editing.

Serial No.	Precision genetic approaches	Trait	Transgene/target gene	Key finding	Reference
1.	Transgenic	Disease resistance	Overexpression of the *Hrap* gene from sweet pepper	Enhanced resistance to BXW in the screen house and confined field trials.	(L. Tripathi et al., 2010; L. Tripathi, Tripathi, et al., 2014)
2.	Transgenic	Disease resistance	Overexpression of the *Pflp* gene from sweet pepper	Enhanced resistance to BXW in the screen house and confined field trials.	(Namukwaya et al., 2012; L. Tripathi, Tripathi, et al., 2014)
3.	Transgenic	Disease resistance	Overexpression of *Xa21* gene from rice	Enhanced resistance to BXW in the screen house	(J. N. Tripathi, Lorenzen, et al., 2014)
4.	Transgenic	Disease resistance	Overexpression of stacked genes (*Hrap-Pflp*)	Enhanced disease resistance in the screen house	(Muwonge et al., 2016)
5.	Transgenic	Disease resistance	Overexpression of non-pathogenesis related (NRR) *NH1* gene	Enhanced resistance to BXW and Foc tropical race 1 in the screen house	(J. N. Tripathi, 2018)
6.	Transgenic	Disease resistance	Overexpression of *Arabidopsis–EFR* gene	Enhanced resistance to BXW in the screen house	(Adero et al., 2023)
7.	Transgenic	Disease resistance	Overexpression of *anti-apoptosis* gene	Enhanced resistance to Foc tropical race 1‘ in the screen house	(Paul et al., 2011; Magambo et al., 2016)
8.	Transgenic	Disease resistance	Overexpression of *RGA2* or *Ced9* genes	Enhanced resistance to Foc tropical race 1 in the confined field trial	(J. Dale et al., 2017b)
9.	Transgenic	Disease resistance	Overexpression of rice thaumatin-like protein (TLP) or PR-5	Enhanced resistance to Foc tropical race 1 in the screen house	(Mahdavi et al., 2012)
10.	Transgenic	Disease resistance	Overexpression of rice *chitinase* gene	Enhanced resistance to *Mycosphaerella fijiensis* in the screen house	(Kovács et al., 2013)
11.	Transgenic	Disease resistance	Overexpression by stacking *ThEn-42* gene with *StSy*	Enhanced resistance to *Mycosphaerella fijiensis* in the screen house	(Vishnevetsky et al., 2011)
12.	Transgenic	Disease resistance	*RNAi* gene silencing	Enhanced resistance to BBTV in the screen house	(Elayabalan et al., 2013; Shekhawat et al., 2012)
13.	Transgenic	Disease resistance	*RNAi* gene silencing of virus movement protein	Enhanced resistance to BBTV in the screen house	(Jekayinoluwa, 2020)
14.	Transgenic	Disease resistance	RNAi silencing of *Acetylcholinesterase* gene in *Pentalonia nigronervosa*	Enhanced resistance to BBTV in the screen house	(Jekayinoluwa et al., 2021)
15.	Transgenic	Pest resistance	Overexpression of *rice cystatin* gene	Enhanced resistance to nematodes in the screen house	(Atkinson et al., 2004)
16.	Transgenic	Pest resistance	Overexpression of antifeedant cysteine proteinase inhibitor and synthetic peptide	Enhanced resistance to nematodes in the screen house	(Roderick et al., 2012)

(Continues)

TABLE 1 (Continued)

Serial No.	Precision genetic approaches	Trait	Transgene/target gene	Key finding	Reference
17.	Transgenic	Pest resistance	Overexpression of antifeedant cysteine proteinase inhibitor and synthetic peptide	Enhanced resistance against *Radopholus similis* and *Helicotylenchus multicinctus* nematodes	(L. Tripathi, Babirye, et al., 2015)
18.	Transgenic	Pest resistance	Overexpression of rice and papaya cysteine proteinase inhibitors	Enhanced resistance against weevils	(Kiggundu et al., 2010)
19.	Transgenic	Biofortification	Overexpression of banana/maize phytoene synthase gene 1	Increased level of provitamin A in the transgenic banana	(Paul et al., 2017)
20.	CRISPR/Cas9 genome editing	Disruption of chlorophyll production	*Phytoene desaturase* (*PDS*) gene	Mutant events exhibited albino phenotype	(Kaur et al., 2018; Naim et al., 2018; Ntui et al., 2020)
21.	CRISPR/Cas9 genome editing	Disruption of chlorophyll production	*RP43/CHAOS39* gene	Mutant events displayed pale-green phenotypes	(Zorrilla-Fontanesi et al., 2020)
22.	Cas-Clover genome editing	Disruption of chlorophyll production	*Phytoene desaturase* (*PDS*) gene	Mutant events exhibited albino phenotype	(L. Tripathi et al., 2023)
23.	CRISPR/Cas9 genome editing.	Biofortification	Editing of *lycopene epsilon-cyclase* (*LCYε*) gene	Mutant events accumulated high levels of beta-carotene	(Kaur et al., 2018)
24.	CRISPR/Cas9 genome editing	Plant architecture	*Gibberellin 20ox2* (*MaGA20ox2*) gene	Edited plants exhibited reduced plant height	(Shao et al., 2020)
25.	CRISPR/Cas9 genome editing	Plant architecture	*Musa aminocyclopropane-1-carboxylase oxidase* (*MaACO1*) gene	Edited plants exhibited reduced plant height	(Hu et al., 2021)
26.	CRISPR/Cas9 genome editing	Disease resistance	*Downy mildew resistance 6* (*DMR6*)	Enhanced resistance against BXW in the screen house	(J. N. Tripathi et al., 2021)
27.	CRISPR/Cas9 genome editing	Disease resistance	*Endogenous banana streak virus (eBSV)*	Resistance against BSV in the screen house	(J. N. Tripathi, Ntui, Ron, et al., 2019)

Abbreviations: BBTV, banana bunchy top virus; BSV, banana streak virus; BXW, banana Xanthomonas wilt.

develop BBTV-resistant bananas through genetic engineering. In greenhouse evaluations, transgenic Gonja Manjaya bananas expressing an RNAi construct targeting the movement protein of BBTV exhibited enhanced resistance against the virus (Jekayinoluwa, 2020).

Another approach we adopted was to control the vector transmitting the disease. By employing RNAi-mediated silencing of the *Acetylcholinesterase* (AChE) gene in *Pentalonia nigronervosa*, the banana aphid, researchers aimed to inhibit the aphid population by potentially reducing the virus transmission (Jekayinoluwa et al., 2021). AChE is an essential enzyme responsible for the hydrolytic metabolism of the neurotransmitter acetylcholine in animals and insects. In this study, the *AChE* gene of the banana aphid was targeted for silencing by RNAi through transgenic expression of AChE dsRNA in banana and plantain plants. The transgenic banana cultivar Cavendish Williams and plantain cultivars Gonja Manjaya and Orishele expressing AChE dsRNA showed a significant reduction in aphid populations growing on the transgenic plants compared to those raised on control plants, demonstrating the potential of this RNAi-based strategy for managing aphids and curbing disease transmission (Jekayinoluwa et al., 2021).

To tackle BXW disease, the researchers at IITA have introduced defense genes from other plant species into the banana genome. For instance, the *Hypersensitivity response-assisting protein* (*Hrap*) gene from sweet pepper (*Capsicum annuum*) has been successfully integrated into the genome of bananas to confer resistance against BXW disease (L. Tripathi et al., 2010). The *Hrap* gene, a plant defense gene, enhances hypersensitive cell death, an essential defense mechanism in plants (Chen et al., 2000). The *Hrap* gene is widely distributed throughout a broad range of plant species, including tobacco, *Arabidopsis*, and rice.

Similarly, the transgenic banana overexpressing the *Plant ferredoxin-like protein* (*Pflp*) gene demonstrated enhanced resistance to BXW disease (Namukwaya et al., 2012). *The Pflp* gene plays a crucial role in plant defense, producing

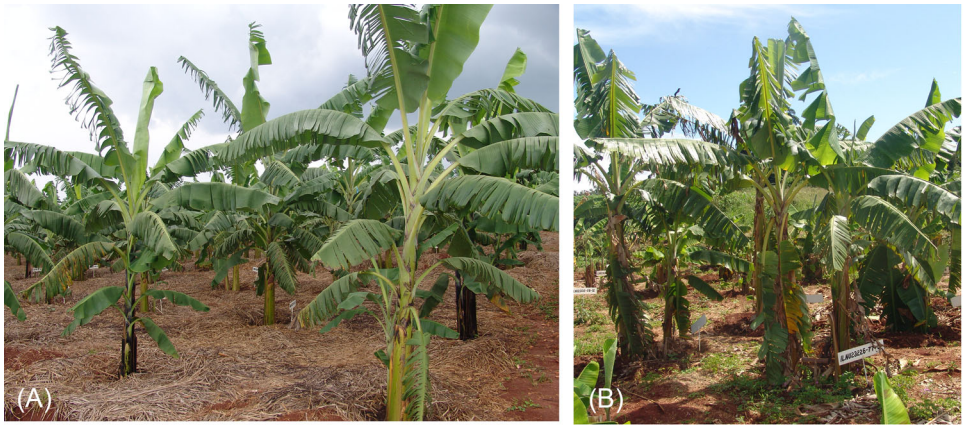

FIGURE 1 Confined field trials of transgenic bananas resistant to banana Xanthomonas wilt (BXW) disease (A) and nematodes (B) at National Agriculture Research Laboratory (NARL), Kawanda, Uganda.

active oxygen species and induction of hypersensitive response by bacterial pathogens (Dayakar et al., 2003; Huang et al., 2004). Transgenic banana of cultivar Sukali Ndiizi harboring sweet pepper *Pflp* gene was evaluated under greenhouse and showed enhanced resistance against the bacterial pathogen Xcm (Namukwaya et al., 2012).

Further, the transgenic bananas overexpressing *Hrap* or *Pflp* gene were evaluated in the confined fields in Uganda for up to three generations. Several of the transgenic events showed complete resistance against Xcm and retained the resistance even in the ratoon crop, which suggests that the transgenic approach could provide a lasting solution to farmers for controlling BXW (Figure 1A) (L. Tripathi, Tripathi, et al., 2014). There is a risk of breaking down resistance in transgenic plants overexpressing a single gene. To avoid this situation, researchers at IITA have generated transgenic banana by stacking *Hrap-Pflp* genes. The transgenic banana expressing stacked *Hrap* and *Pflp* genes showed complete resistance to BXW similar to individual genes (Muwonge et al., 2016). In addition, stacking transgenes will provide durable resistance. Extensive evaluations using published information on the history of safe use of the natural source of the proteins as well as established bioinformatics sequence comparison methods to known allergens and toxins have demonstrated the safety of the expressed proteins HRAP and PFLP, indicating no potential risks of allergenicity or toxicity (Jin et al., 2017). Furthermore, the expression of *Hrap* and *Pflp* genes did not affect nontarget rhizobacteria and endophytes in transgenic banana (Nimusiima et al., 2015).

There are two types of immune receptors, race-specific ephemeral known as "NBS-LRR" receptors and pattern-recognition receptors (PRRs), mainly used in breeding programs. Both are required for the fitness of plants. The rice Xa21, a PRR showed broad-spectrum resistance against *Xanthomonas oryzae* pv. *oryzae*, the causative agent of rice bacterial blight (Ronald et al., 1992; Wang et al., 1996). Based on the assumption of broad-spectrum resistance by inter-generic transfer of PRR, researchers transferred the rice *Xa21* gene into the banana cultivar Gonja Manjaya. The transgenic banana overexpressing the *Xa21* gene showed complete resistance to *Xcm* compared to control non-transgenic plants (J. N. Tripathi, Lorenzen, et al., 2014).

Further, transgenic banana overexpressing a non-pathogenesis-related *NH1* gene also showed enhanced resistance to BXW (J.N. Tripathi, 2018). Researchers have also explored elongation factor receptors related (*EFR*) gene isolated from *Arabidopsis thaliana* (Adero et al., 2023). The banana cultivar Cavendish overexpressing the *AtEFR* gene was generated and evaluated for resistance against Xcm under greenhouse conditions. The transgenic banana events exhibited 33%–76% resistance to BXW disease compared to the non-transgenic control plants. This study suggests that the function of *AtEFR* is retained in bananas with the potential of enhancing the resistance against Xcm under field conditions (Adero et al., 2023).

In the realm of pest resistance, transgenic approaches have been explored to combat plant-parasitic nematodes. Researchers developed nematode-resistant plantain Gonja Manjaya by introducing maize cystatin that inhibits nematode digestive cysteine proteinases and a synthetic peptide that disrupts nematode chemoreception (Roderick et al., 2012). These transgenic plantains exhibited significant resistance to *Radopholus similis* and *Helicotylenchus multicinctus*. A confined field trial further demonstrated that transgenic overexpression of an antifeedant cysteine proteinase inhibitor and synthetic peptide showed enhanced resistance against *Radopholus similis* and *Helicotylenchus multicinctus* nematodes (Figure 1B) (L. Tripathi, Babirye, et al., 2015). Similarly, the transgenic banana Cavendish Williams overexpressing rice cystatin conferred resistance against *Radopholus similis* under greenhouse conditions (Atkinson et al., 2004). Moreover, the rice and papaya cysteine proteinase inhibitors seem promising in resisting the banana weevil (*Cosmopolites sordidus*), a destructive pest (Kiggundu et al., 2010).

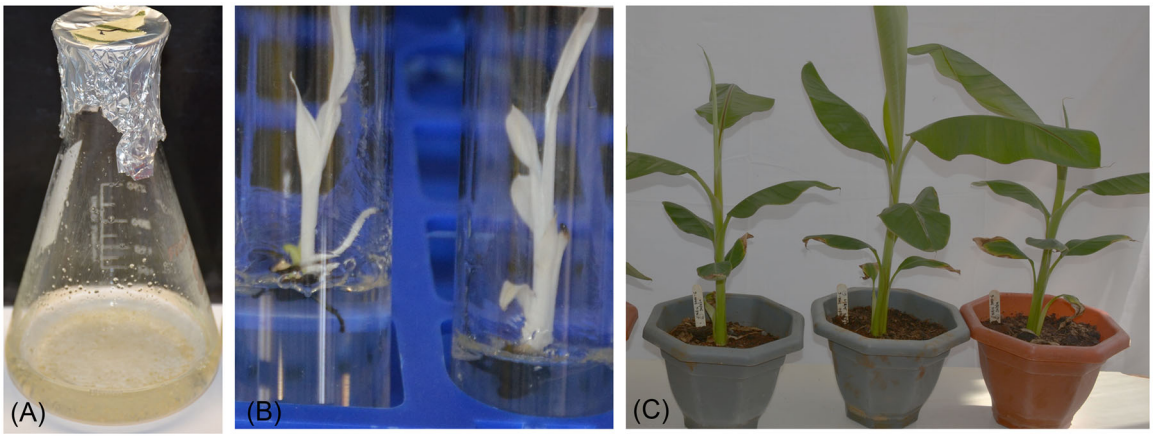

FIGURE 2 Genome edited of banana. (A) Embryogenic cell suspension used for delivering of CRISPR/Cas9 reagents, (B) albino plantlets of genome edited banana with disruption of *phytoene desaturase* (*PDS*) gene, and (C) genome edited disease resistance banana with editing of *Musa* ortholog of *Downy mildew resistance-6* (*MusaDMR6*) gene.

Vitamin A deficiency is a major public health problem, especially in children and pregnant and lactating mothers in developing countries. Some efforts have been made to develop biofortified banana, also called golden banana, using transgenic approaches (Paul et al., 2017). Researchers developed provitamin A-enriched golden banana using transgenes (phytoene synthase genes) from banana as well as maize. These biofortified transgenic bananas have fivefold more provitamin A compared to control non-transgenic ones.

3.3 | Improving banana through genome editing

The production of banana is facing numerous biotic and abiotic challenges, leading to significant losses. To combat production constraints, there is an urgent need to explore scientific innovations, particularly new breeding technology like genome editing. Although commercially available genome-edited bananas are not yet in the market, researchers are actively working on developing bananas with improved traits using genome editing techniques (Table 1).

Genome editing technologies have successfully been developed for bananas in various laboratories. For instance, Kaur et al. (2018) demonstrated genome editing in banana in the cultivar Rasthali (AAB genome) by targeting the *phytoene desaturase* (*PDS*) gene. The authors used a single gRNA to generate mutations in the *PDS* gene, resulting in albino phenotypes but with a mutation efficiency of 59%. The *PDS* gene is involved in the carotene biosynthetic pathway, and its mutation produces albino plants, which are easily identifiable. In a similar study, Naim et al. (2018) reported the editing of the *PDS* gene in Cavendish Williams (AAA genome) with 100% efficiency using polycistronic gRNAs. At IITA, researchers have established a robust genome editing tool for bananas and plantains by targeting *PDS* gene (Ntui et al., 2020). They delivered a CRISPR/Cas9 construct containing two gRNAs into embryogenic cells of the Sukali Ndiizi banana cultivar and the Gonja Manjaya plantain cultivar (Figure 2A). The resulting mutants displayed albino phenotypes, indicating disruptions in the function of the PDS gene (Figure 2B). Sequence analysis confirmed the presence of indels (insertions/deletions) in the targeted sites, demonstrating a mutation efficiency of 100%.

PDS knockout negatively impacts plant development; the plants usually die because they cannot photosynthesize. To mitigate the negative impact of PDS knockout on plant development, Zorrilla-Fontanesi et al. (2020) modified the *RP43/CHAOS39* gene, which encodes the chloroplast signal recognition particle (cpSRP) machinery, to serve as a visual marker. The CHAOS39-modified banana plants displayed pale green phenotypes and grew normally. It is essential to exercise caution when using cpSRP43/CHAOS39 as a visual marker, as the pale green phenotype can also result from other factors.

The CRISPR/Cas9 tool has now been applied for banana improvement. For example, Kaur et al. (2018) used CRISPR/Cas9 technology to increase β-carotene content in the Cavendish cultivar Grand Naine by editing the *lycopene epsilon-cyclase* gene. The edited bananas had enhanced accumulation of β-carotene content up to sixfold (~24 μg/g) in the fruit pulp compared to the unedited plants. Further, the *gibberellin 20ox2* (*MaGA20ox2*) gene was edited in the Gros Michel banana cultivar, resulting in plants with reduced height (Shao et al., 2020). The *aminocyclopropane-1-carboxylase oxidase* gene was also edited in the *Musa acuminata* (AAA group, cv. Brazilian), leading to plants with shorter heights and delayed ripening (Hu et al., 2021).

As an alternative to the CRISPR/Cas9, researchers developed a different genome-editing technique for banana using the Cas-CLOVER system (L. Tripathi et al., 2023). Cas-CLOVER is a dual-guide RNA system that, when dimerized

with nuclease Clo051, produces double-strand breaks (Madison et al., 2022). The Clo051 endonuclease serves as a binding protein on the target site in the genome of any organism, and the fusion protein consists of a nuclease inactivated or dead Cas9 (dCas9) protein. The Cas-CLOVER endonuclease system uses two guide RNAs (gRNAs) in addition to the Clo051 nuclease activity, which necessitates dimerization of subunits associated with each guide RNA, as opposed to employing a single gRNA, as in CRISPR. The Cas-CLOVER genome editing system is very targeted and precise because Clo051 only creates double strands when both gRNAs are dimerized. L. Tripathi et al. (2023) developed genome-edited banana carrying mutations of the *PDS* gene using this technique. The mutant events displayed albino phenotype, indicating disruption of the *PDS* gene.

3.4 | Application of CRISPR/Cas9 technology to develop disease-resistant banana

CRISPR/Cas-based genome editing advancements have opened up new possibilities for accelerating banana improvement. With the availability of well-annotated reference genome sequences, a CRISPR/Cas9-editing system, and a well-developed banana transformation and regeneration system, researchers can now develop disease-resistant banana varieties by precisely editing the plant's endogenous genes (L. Tripathi et al., 2020; L. Tripathi et al., 2022; L. Tripathi, Tripathi, et al., 2019). One potential application of gene editing in banana is developing varieties resistant to diseases such as Xanthomonas wilt. By editing genes that make the plant susceptible to the disease, scientists hope to create more resistant plants.

An example of a gene targeted for editing to develop gene-edited disease-resistant bananas is *downy mildew resistance 6* (*DMR6*), a susceptibility gene encoding 2-oxoglutarate Fe (II)-dependent oxygenase that is upregulated during Xcm infection (J. N. Tripathi et al., 2021). DMR6 and its paralog DMR6-like oxygenase1 play a key role in suppressing plant immunity and are overexpressed during pathogen infection (Zeilmaker et al., 2015). By delivering the CRISPR/Cas9 reagents targeting the editing of *MusaDMR6* into the ECS of Sukali Ndiizi, researchers generated mutants with enhanced resistance against Xcm, as confirmed through greenhouse evaluations (Figure 2C; J. N. Tripathi et al., 2021). The banana mutants did not exhibit any detrimental effect on plant growth. These mutants need to be tested in field conditions to assess the overall agronomic performance.

Although genome-edited bananas are still in the research phase and have not reached farmers yet, they hold promise for providing a sustainable and effective solution to the problem of BXW in banana crops. The introduction of resistance to this devastating disease could lead to significant improvements in yield and economic viability for farmers, benefiting both farmers and consumers who depend on this important food crop.

In addition to CRISPR/Cas9 technology, CRISPR/Cas activation can complement classical breeding in developing resistance to BXW disease. By using a modified version of the Cas9 enzyme, known as dCas9, the expression of single or multiple endogenous genes can be upregulated without introducing double-stranded breaks in the target gene. Researchers at IITA are progressing in developing resistance to BXW disease by upregulating multiple endogenous defense genes in susceptible banana cultivars.

In an effort to combat BSV, researchers at IITA have investigated the possibility of inactivating the eBSV by knocking out the integrated sequences in the B genome of the farmer-preferred plantain cultivar Gonja Manjaya (J. N. Tripathi, Ntui, Ron, et al., 2019). Through targeted mutations, the researchers were able to disrupt the formation of functional episomal BSV proteins. As a result, 75% of the mutants showed no symptoms compared to the non-edited plants under stress conditions in the greenhouse, confirming the inactivation of eBSV. This study has the potential to develop virus-free banana germplasm to improve the B genome and its derived genotypes for the development of improved hybrid bananas that can be disseminated globally (J. N. Tripathi, Ntui, Ron, et al., 2019).

3.5 | Challenges with CRISPR technology in banana

One of the challenges of using CRISPR technology in bananas is the integration of foreign gene sequences into the plant's genome. The transgene-free genome-edited plants, which do not contain any foreign DNA integration like selection markers, promoters, terminators, or border sequences are treated similarly to mutants developed through classical breeding in several countries. These transgene-free gene-edited plants are assumed to be safer for consumption and more likely to pass biosafety regulations in countries with genome editing regulatory guidelines.

While the removal of integrated foreign gene sequences through genetic segregation is possible in sexually propagated crops, such as cereal crops, it is more challenging in clonally propagated crops like bananas (Nadakuduti et al., 2018). The development of efficient strategies for producing foreign DNA-free plants is a significant challenge. Surmountable progress has been made in optimizing CRISPR/Cas9 delivery into banana, particularly for targeted gene editing. However, many significant challenges remain, particularly in developing effective strategies for producing foreign DNA-free genome-edited bananas. Three different methods have been proposed to develop foreign DNA-free genome-edited plants. The first method involves segregation of foreign gene integration through backcrossing or selfing the mutants. This method

is mainly applied to sexually propagated crops. The second method utilizes CRISPR/Cas9 ribonucleoproteins (RNPs) that can be delivered to plant cells by particle bombardment or polyethylene glycol (PEG) transfection of protoplasts. The RNPs are quickly degraded by the endogenous cell proteases immediately after editing the gene. The third method involves the excision of CRISPR/Cas machinery after achieving the desired edits. In IITA, researchers are exploring the possibility of producing foreign DNA-free banana mutants resistant to BXW using RNPs or excision of the CRISPR/Cas reagents.

Another challenge in utilizing CRISPR technology in crops like banana is their polyploid nature and highly heterozygous genome. Mutating multiple alleles simultaneously is difficult, and it requires the analysis of numerous mutants to obtain perfectly edited mutants with multiallelic mutations (Nadakuduti et al., 2018; Ntui et al., 2020).

4 | FUTURE PERSPECTIVE

One of the greatest challenges in agriculture is ensuring food security for the rapidly growing human population, estimated to reach more than 10 billion in a few years compared to 7.9 billion in July 2023 (United Nations, Department of Economic and Social Affairs, 2022). Therefore, there is an urgency to increase food production of staple crops. In Africa, the focus should be on bananas, as they are essential for food security and income generation. Investing in precision genetics for banana holds potential in addressing these challenges and feeding more people per hectare area of production compared to cereal crops (L. Tripathi, Tripathi, et al., 2019).

Genome editing can be applied to improve disease resistance and yield potential in bananas as well as enhance their nutritional content. Additionally, specific traits related to plant morphology, such as reducing plant height, can be improved through genome editing. One approach to overcome biosafety regulatory hurdles is integrating edited parents or diploid progenitor bananas into the breeding program for further improvement. The transgenes can be removed through segregation, resulting in transgene-free final products. Some progress has been made in banana and other clonally propagated crops to deliver the CRISPR-Cas9 protein-gRNA RNPs complexes into the embryogenic cells, which edit the target sites upon delivery and are subsequently degraded by the plant's endogenous proteases (J.N. Tripathi et al., 2019). This foreign DNA-free approach can be instrumental in developing disease-resistant banana varieties by targeting multiple genes simultaneously. In many countries, edited plants without foreign DNA require less stringent regulatory approval, making their commercialization more favorable than transgenic crops (Razzaq et al., 2019). Wu et al. (2020) established a PEG-mediated banana protoplast transformation system using this approach. They successfully delivered CRISPR/Cas9 and CRISPR/Cas12a plasmids and CRISPR/Cas9 RNPs targeting the *PDS* gene into protoplasts of banana cultivar Cavendish. However, the editing efficiency using CRISPR/Cas9-RNPs was much lower than that of the CRISPR/Cas9 plasmid.

Recent advancements in morphogenic gene technology have facilitated the transformation of recalcitrant cultivars as the current transformation method is cultivar dependent. Morphological regulator genes such as *WUSCHEL2* (*Wus2*), *Baby boom* (*Bbm*), (Lowe et al., 2016), and *SHOOT MERISTEMLESS* (*Stm*) (Scofield et al., 2014) seem promising in transforming many recalcitrant plant species. These genes can make the transformation of recalcitrant banana cultivars, particularly cooking varieties such as Ngombe and Uganda Green more accessible. Developing transformation protocols using these morphogenic genes will facilitate the editing of previously difficult-to-transform banana cultivars.

5 | CONCLUSION

The development of disease-resistant and high-yielding banana varieties is crucial for ensuring food security and income generation for smallholder farmers. The utilization of genetic transformation techniques, such as *Agrobacterium*-mediated approaches, has allowed for the successful introduction of desirable traits and enhanced resistance against various diseases and pests. Transgenic banana events have shown promising results in controlled environments and field evaluations. Continued research, field evaluations, and regulatory approvals are necessary to ensure these technologies' safety, efficacy, and acceptance.

The emergence of CRISPR/Cas-based genome editing technology has opened new possibilities for precisely editing the endogenous genes of bananas. This technology offers the potential to develop disease-resistant bananas by introducing specific mutations into genes that make the plant susceptible to pathogens. Although genome-edited banana plants are still in the research phase and subject to regulatory oversight, they hold promise for addressing disease susceptibility and improving crop quality.

The challenges associated with CRISPR technology in bananas include the integration of foreign gene sequences, the production of transgene-free edited plants, and the complexities of the polyploid and heterozygous nature of the banana genome. However, researchers are making significant progress in developing transgene-free plants and exploring strategies to overcome these challenges. By addressing the challenges and harnessing the full potential of precision genetics, we can meet the demand for increased food production and secure the future of banana cultivation.

Integrating genome editing technology with conventional breeding programs and utilizing foreign DNA-free approaches can further enhance the potential for banana

improvement. By combining the advantages of genome editing with the existing breeding efforts, researchers can develop disease-resistant, high-yielding banana varieties with improved nutritional content.

AUTHOR CONTRIBUTIONS

Jaindra Nath Tripathi: Writing—original draft; writing—review and editing; figure preparation. **Valentine Otang Ntui**: Writing—original draft; writing—review and editing. **Leena Tripathi**: Conceptualization; funding acquisition; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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There are no original data associated with this article.

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