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REVIEW

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Application of genetic modification and genome editing for developing climate-smart banana

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Abstract

Banana is a major staple food crop feeding more than 500 million people in tropical and subtropical countries. Its production is largely constrained by diseases and pests in addition to other factors such as declining soil fertility, narrow genetic diversity in germplasm, and inadequate availability of clean planting material. The impact of climate change, particularly a rise in temperature and drought, is predicted to affect production adversely due to direct effect on plant agronomy and also influence on pathogens, pests, and their interactions with host plants. There is need to develop climate-smart varieties of banana with multiple and durable resistance to combat abiotic stresses such as extreme temperature and drought, and biotic stresses such as pathogens and pests. Modern breeding tools, including genetic modification and genome editing, can be applied for the improvement of banana bypassing the natural bottlenecks of traditional breeding. Intensive efforts using genetic modification have been made to develop improved banana varieties with resistance to biotic stresses; however, these need to be coupled with tolerance to abiotic stresses. Genome editing, an emerging powerful tool, can be applied for developing sustainable solutions to adapt to climate change by resisting biotic and abiotic stresses. CRISPR/Cas9-based genome editing has been lately established for banana, paving the way for functional genomics allowing identification of genes associated with stress-tolerant traits, which could be used for the improvement of banana for adaptation to a changing climate. This article presents an overview of recent advancements and prospective on the application of genetic modification and genome editing for developing climate-smart banana.

KEYWORDS

banana, climate smart, genetic engineering, genome editing, improved varieties

1 | INTRODUCTION

Banana (*Musa* spp.) including plantain is one of the major staple food crops grown in over 140 countries in the subtropics and tropics with annual production worldwide of around 148 million metric, feeding about 500 million people (FAOSTAT, 2016). It is a valuable food security and cash crop as it can be cultivated in diverse environments and produces fruits throughout the year in these favorable weather conditions (Jones, 2000). Smallholder farmers mainly cultivate

banana for domestic consumption and local or regional markets; only about 15% of production enters international markets. Africa contributes one-third of the world's production with East Africa being the biggest banana-growing region accounting for about 40% of total production in Africa. East African countries such as Burundi, Rwanda, and Uganda annually consume the highest amount of banana at 220–460 kg per person annually (Kilimo Trust, 2012). Based on food security data, banana provides 30%–60% of the daily per capita calorie intake in these countries (Abele & Pillay, 2007). Bananas are full of minerals, vitamins, and carbohydrate and are considered one of the main sources of energy for millions of people in East Africa.

Hundreds of cultivars of bananas are grown and consumed worldwide, but large-scale farmers mainly grow the Cavendish type of dessert bananas for commercialization in local and international markets. However, plantain is grown largely in Central and West Africa, and East African Highland banana (EAHB) is cultivated in East Africa. Other dessert banana varieties such as Sukali Ndiizi and Gros Michel are also grown at minor level in Africa.

Banana production is seriously affected by several factors, specifically biotic and abiotic stresses, declining soil fertility, narrow genetic diversity in germplasm, and inadequate availability of clean planting material among smallholder farmers. Diseases and pests are one of the major factors limiting yields worldwide. The production is mainly reduced by many bacterial, fungal, and viral pathogens, mainly *Xanthomonas campestris* pv. *musacearum* (Xcm) causing banana Xanthomonas wilt (BXW), *Ralstonia solanacearum* causing moko and bugtok disease, *Ralstonia syzygii* subsp. *celebesensis* causing blood disease, *Pseudocercospora fijiensis, P. musae,* and *P. eumusae* causing black Sigatoka, yellow Sigatoka, and leaf spot disease, respectively, *Fusarium oxysporum* f. sp. *cubense* causing fusarium wilt (commonly known as panama disease), and viruses such as *banana bunchy top virus* (BBTV) and *banana streak virus* (BSV), and pests such as nematodes and weevils (Jones, 2000; Ploetz, 2015; Tripathi, Tripathi, & Kubiriba, 2016; Tripathi et al., 2009; Tushemereirwe, Kangire, & Ssekiwoko, 2004). There is a huge yield gap in banana production in the areas where several of these pathogens and pests are present together. Some of these diseases are wiping out banana from the infected fields.

In addition, changes in climate and weather are also having a significant impact on banana yields, particularly in the regions where the crop is grown with minimal or no irrigation. For example, banana yields were reported to be affected by variation in temperature and rainfall in Uganda (Sabiiti et al., 2016), which is the largest producer and consumer of banana. Drought is a major factor for yield losses, mainly in rain-fed fields in Uganda (Sabiiti et al., 2016; Van Asten, Fermont, & Taulya, 2010). The effects of climatic extremes on banana yields depend on the stage of the crop at the time, as well as the frequency and duration of exposure of the crop to the extreme climate (Van Asten et al., 2010). Strategies need to be developed for banana production to adapt to extreme changes in climate, particularly rainfall and temperature in the banana-growing areas.

Extreme climate is predicted to have harmful influences not only on plant agronomic traits but also on the soil fertility, and pathogens and pests, which affect crop productivity (Dhanker & Foyer, 2018). The human population of the world is projected to reach 9.8 billion in 2050 and 11.2 billion by 2100 (United Nations, Department of Economic and Social Affairs, Population Division, 2015). There is a pressing need to close the yield gap in staple crops and enhance food production in order to feed the world. As banana is one of the main staple food crops for Africa, emphasis should be on banana rather than on cereals, which is the situation in many other parts of the world. Investment in banana improvement holds great potential for improving food security as these crops feed more people per unit area of production than other staple crops (West et al., 2014).

To fulfill the increasing demand for food with the same or limited resources, better and more efficient ways to produce food are required. One option is to utilize modern breeding tools such as genetic modification and genome editing for crop improvement. Currently, intensive efforts are underway to increase yields of banana through developing improved varieties with resistance/tolerance to biotic and abiotic stresses. This article presents an overview of recent progresses and prospective on the application of genetic modification and genome editing for developing climate-resilient banana.

2 | IMPROVEMENT OF BANANA FOR CLIMATE-RESILIENT TRAITS

Climate change including extreme temperature and drought is not only having negative impact on the agronomic conditions of banana plants but also expected to impact soil nutrients, pathogens, and pests. Therefore, there is need to develop climate-resilient varieties with broad spectrum and durable resistance to both biotic and abiotic stresses.

3 | GENETIC MODIFICATION AND GENOME EDITING

Developing improved varieties of banana using conventional breeding is challenging because of the low genetic variability in *Musa* germplasm, polyploidy, lengthy production cycle, and sterility of majority of the cultivars commonly grown by farmers (Silva et al., 2001). Genetic engineering is a very

effective tool, which allows the transfer of useful traits from different species or across the same species, bypassing natural bottlenecks of breeding, thus making it applicable for the improvement of banana.

It provides a further option for the development of improved varieties resistant to diseases, particularly when no host plant resistance is available among banana germplasm. Although no genetically modified (GM) banana and plantain have yet been released for commercial purposes, their production should be encouraged and supported through scientific knowledge and risk assessment studies.

Precise genome editing is the new genetic engineering tool for crop improvement. Several techniques such as zinc finger nucleases (ZFNs) (Maeder et al., 2008; Sander et al., 2011), TAL effector proteins (TALENs) (Christian et al., 2010; Li et al., 2011; Miller et al., 2011), RNA-guided nucleases (RGENs), and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) (Cong et al., 2013; Jinek et al., 2012; Mali et al., 2013) have been developed for targeted genome editing in plants. All these methods are based on the formation of double-stranded breaks at specific loci and in triggering DNA repair mechanism (Weinthal & Gürel, 2016). CRISPR/Cas9 has emerged as a potent genome editing tool that can be used efficiently to induce targeted mutations in the genomes of plant species to produce improved varieties.

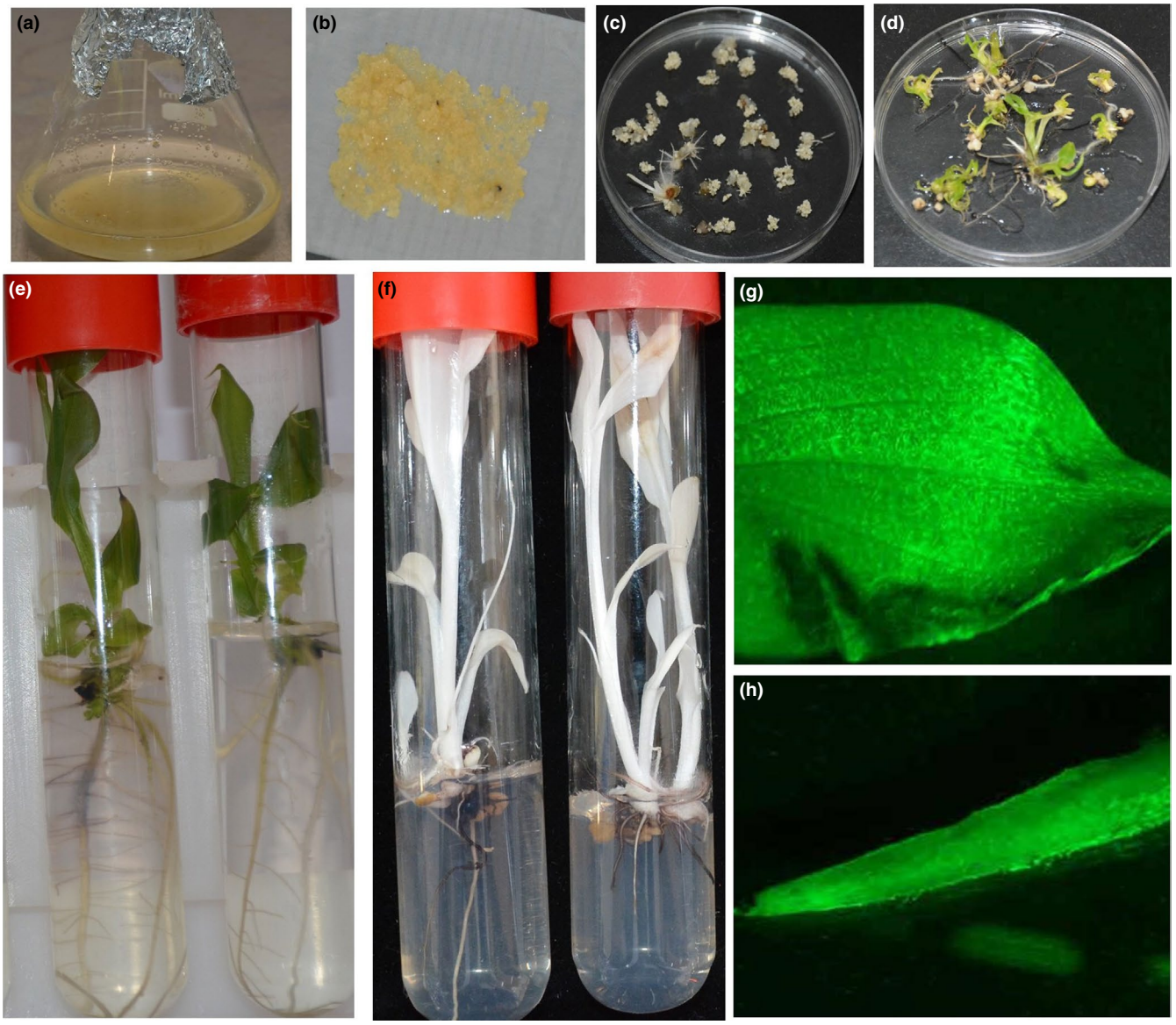

FIGURE 1 Genetic transformation and regeneration of banana. (a) Embryogenic cells of banana used as explant for transformation, (b) *Agrobacterium*-infected embryogenic cells, (c) transformed embryogenic cells on selective regeneration medium, (d) embryos germinating on selective regeneration medium, (e) complete plantlet of transgenic banana regenerated on selective medium, (f) genome-edited plant with mutations in *phytoene desaturase* (*PDS*) showing albino phenotype, and (g–h) transgenic plant showing expression of green florescent protein in leaf and root under UV-light source

This technology has been successfully applied in many organisms including several plant species (Scheben, Wolter, Batley, Puchta, & Edwards, 2017). It has not only been established for model plants such as *Arabidosis thaliana* and *Nicotiana benthamiana* but also been established for complex crops such as rice, wheat, maize, sorghum, tomato, soybean, apple, citrus, poplar, and coffee (Breitler et al., 2018; Song et al., 2016). Most of the genome editing is reported in the seed crops; however, the CRISPR/Cas9-based genome editing has recently also been reported in vegetatively propagated crops including potato, cassava, and banana with mutations in *phytoene desaturase* (*PDS*) (Butler, Baltes, Voytas, & Douches, 2016; Kaur et al., 2017; Naim et al., 2018; Odipio et al., 2017).

Kaur et al. (2017) demonstrated genome editing of banana cultivar "Rasthali" (AAB) by creating mutations in the *PDS* gene, a key enzyme in the carotene biosynthesis pathway, leading to an albino phenotype. They used a single gRNA and obtained mutation efficiency of 59%. Later, higher editing efficiency (100%) of the *PDS* gene was reported in "Cavendish Williams" (AAA) using polycistronic gRNAs (Naim et al., 2018). Similarly, high mutation efficiency was also obtained in our laboratory using multiple gRNAs targeting the *PDS* gene (Figure 1). Establishment of CRISPR/Cas9 system has paved the way for the application of genome editing for the improvement of banana. Multiplexing of CRISPR/Cas9 system can result in editing two or more genes at the same time; so far, a maximum of eight genes have been edited simultaneously in rice (Xie, Minkenberg, & Yang, 2015).

4 | DELIVERY OF PLASMID CONSTRUCTS AND CRISPR REAGENTS TO PLANT CELLS

Developing genetically modified and genome-edited banana requires a highly efficient system for transformation and regeneration (Tripathi, Oduor, & Tripathi, 2015; Tripathi, Babirye et al., 2015). The biggest challenge in the application of CRISPR/Cas9 technology in several crops is the regeneration and genetic transformation of complete plants from transformed cells (Altpeter et al., 2016). Banana has been modified mainly using either *Agrobacterium*-mediated transformation or microprojectile bombardment. *Agrobacterium*-mediated transformation protocols have also been established for many cultivars of banana and plantains (Ganapathi et al., 2001; Khanna, Becker, Kleidon, & Dale, 2004; Tripathi, Mwaka, Tripathi, & Tushemereirwe, 2010; Tripathi, Muwonge, & Tripathi, 2012; Tripathi, Oduor, & Tripathi, 2015). Protocols for microprojectile bombardment technique are available for modifying banana and plantain (Becker, Dugdale, Smith, Harding, & Dale, 2000; Sagi et al., 1995).

Embryogenic cells are the preferred starting material for genetic transformation of banana. However, the generation of embryogenic cells is cultivar-dependent, time-consuming, and laborious (Tripathi, Oduor, & Tripathi, 2015). Many laboratories have tried to develop embryogenic cells from various cultivars of banana using different types of explants such as leaf sheaths, rhizome, shoot tips, zygotic embryos, male flowers, and multiple buds (Becker et al., 2000; Cote et al., 1996; Dheda, Dumortier, Panis, Vuylsteke, & De Langhe,

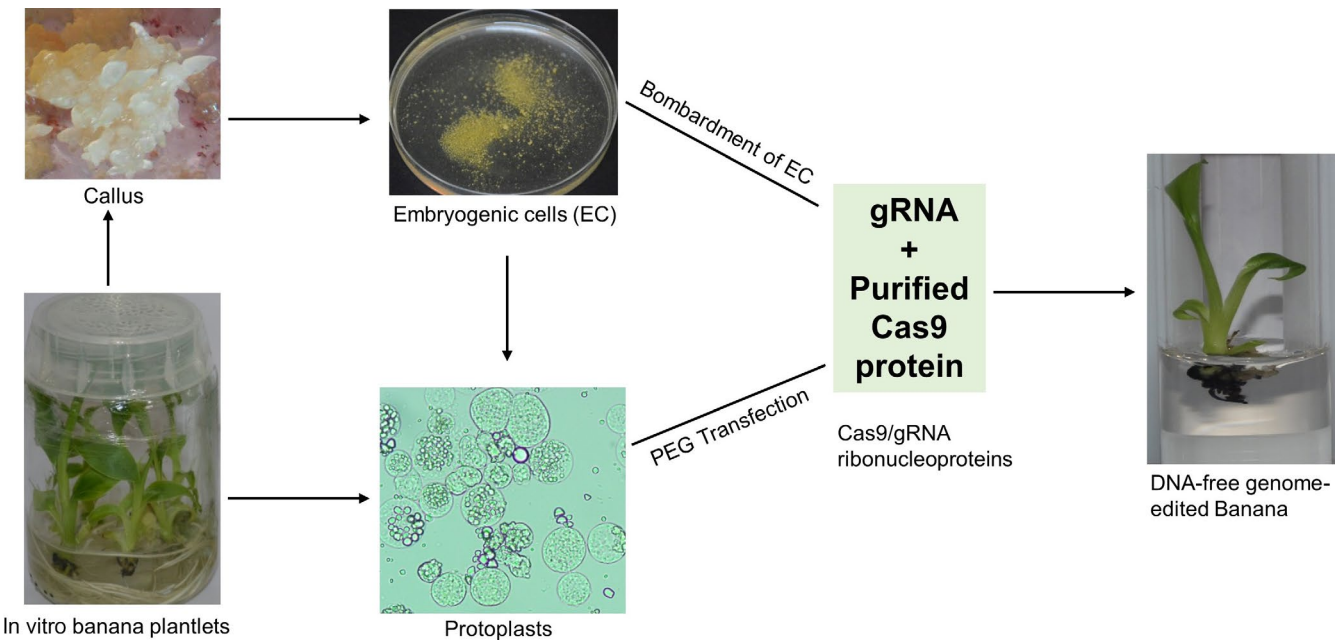

FIGURE 2 Schematic diagram showing generation of DNA-free genome-edited banana by delivering preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) directly into embryogenic cells or protoplasts and regeneration into complete plants

1991; Escalant, Teisson, & Cote, 1994; Ganapathi et al., 2001; Navarro, Escobedo, & Mayo, 1997; Novak et al., 1989). Our group has developed embryogenic cells for many cultivars of banana and plantain using either multiple buds (scalps) or male flowers, depending upon the cultivars (Tripathi et al., 2012; Tripathi, Oduor, & Tripathi, 2015).

IITA-Kenya has established a robust genetic transformation platform for banana and plantain using an *Agrobacterium*-mediated system (Tripathi et al., 2012; Tripathi, Oduor, & Tripathi, 2015). Our laboratory has capacity to transform many cultivars of plantains such as Gonja Manjaya, Agbagba, Orishele, and Obino l'Ewai and dessert bananas such as Sukali Ndiizi, Gros Michel, and Cavendish. This platform is routinely used for the generation of genetically modified and genome-edited banana and plantain (Figure 1). Currently, *Agrobacterium*-mediated transformation is the most common method for delivering CRISPR/Cas9 reagents in banana cells and regenerating complete plants (Kaur et al., 2017; Naim et al., 2018; Tripathi et al., 2019).

5 | DNA-FREE GENOME-EDITED BANANA

Genome-edited banana mediated by plasmid delivery may be considered GM at least in the initial stages of development. The plasmids usually contain selection marker genes and are delivered by *Agrobacterium* into the plant cells. The foreign DNA from the plasmid construct integrates into the plant genome, which is later on removed by backcrossing and selection of transgene(s) free events. However, removal of these plasmid-derived DNA sequences through breeding is not feasible for several cultivars of banana particularly the triploids which are sterile; hence, the mutated plants will be considered as GM. Furthermore, transgenes *Cas9* and the selectable marker along with gRNA from the CRISPR/Cas9 plasmid will integrate into the plant genome, which might lead to chimeric mutations owing to the continuous action of editing machinery, gene disruptions, and off-target mutations. These transgenes can even integrate into the targeted sites and decrease the gene editing efficiency. To tackle these concerns, considerable attempts have been made to deliver preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) directly into plant cells (Liang et al., 2017; Malnoy et al., 2016; Svitashev, Schwartz, Lenderts, Young, & Mark Cigan, 2016; Woo et al., 2015). These RNPs directly edit the target sites immediately after delivery and then are rapidly degraded by endogenous proteases in cells, thus reducing off-target effects and leaving no traces of foreign DNA elements (Kanchiswamy, Malnoy, Velasco, Kim, & Viola, 2017; Woo et al., 2015). The RNPs can be directly delivered into plant cells by particle bombardment, electroporation, cell-penetrating peptides, mesoporous silica nanoparticles, or through polyethylene glycol (PEG) into protoplast.

In banana, preassembled RNPs targeting different traits (abiotic and biotic stresses) for adaptation to different climatic conditions could be coated on the gold particles and delivered to embryogenic cells of banana by particle bombardment or can be delivered to protoplasts through PEG (Figure 2). The embryogenic cells or protoplasts can then be regenerated to full plants. The edited banana plants would withstand environmental stress and bypass GM legislation, as the Cas9 protein-guide RNA complexes will rapidly degrade in the regenerating cell cultures and regenerated plants will be transgene-free.

6 | HOST PLANT RESISTANCE TO PATHOGENS AND PESTS

Changes in climate are predicted to have an impact on pathogen population, survival, life cycle, distribution, host specificity, and susceptibility of host plants (Elad & Pertot, 2014). The pathogens might become more aggressive resulting in an increase in disease incidences and severity on crops. As the effects of climate change will depend on pathosystems and geographical regions, it is necessary that effect of climate change on banana pathogens and their interaction with the host plant should be investigated. No information is available for banana pathosystems except for black Sigatoka disease. It has been reported that *P. fijiensis* could become more aggressive with an increase in temperatures as the growth of the germination tube of spores accelerates with higher temperature (Calberto, Staver, & Siles, 2015). As extreme climate has effects on both banana and pathogens, it is predicted to change disease severity, its distribution, and the economic importance of particular diseases in a given location, and even the number of pathogens challenging banana plantations in the same location. In this scenario, the current disease management system used by farmers for the banana cropping system might not be effective.

Climate change also affects pests and their interaction with host plants (Castex, Beniston, Calanca, Fleury, & Moreau, 2018). Warmer temperature could lead to an increase in pest population and change their distributions (Stange & Ayres, 2010). Therefore, there is urgent need to develop improved varieties with broad spectrum and durable resistance to various diseases and pests. Modern plant biotechnology provides new tools for the development of disease and pest-resistant transgenic banana plants through either overexpression of genes associated with defense or the editing of genes responsible for susceptibility or the negative regulator of the defense pathway. Table 1 lists some of the advances made in developing banana varieties resistant to diseases and pests.

BXW is the biggest challenge for banana production in East and Central Africa (Blomme et al., 2014; Tripathi et al.,

TABLE 1 Summary of advances in the development of genetically modified banana resistant to diseases and pests

Name of gene	Origin of gene	Target pathogens	Mode of action	Resistance		References
				Screen house	Confined field	
Hrap	Sweet pepper	*X. campestris* pv. *musacearum*	Hypersensitivity response	Enhanced resistance	Enhanced resistance	Tripathi et al. (2010), Tripathi, Tripathi et al. (2014)
Pflp	Sweet pepper	*X. campestris* pv. *musacearum*	Hypersensitivity response	Enhanced resistance	Enhanced resistance	Namukwaya et al. (2012), Tripathi, Tripathi et al. (2014)
Stacked *Hrap and Pflp*	Sweet pepper	*X. campestris* pv. *musacearum*	Hypersensitivity response	Enhanced resistance	_	Muwonge et al. (2016)
Xa21	Rice	*X. campestris* pv. *musacearum*	Pathogen recognition receptors induced immunity	Enhanced resistance	_	Tripathi, Lorenzen et al. (2014)
RGA2 or *Ced9*	Banana	*Fusarium oxysporum cubense* tropical race 4	R gene and antiapoptotic mechanism	Enhanced resistance	Enhanced resistance	Dale et al. (2017)
TLP or *PR-5*	Rice	*Fusarium oxysporum cubense* race 1	Defense mechanism	Enhanced resistance	_	Mahdavi et al. (2012)
Antiapoptosis genes *Bcl-xL*, *Ced-9*, *Bcl-2*	Animal	*Fusarium oxysporum cubense* race 1	RNAi	Enhanced resistance	_	Paul et al. (2011)
PhDef1 and PhDef2	Petunia	*Fusarium oxysporum cubense* race 1	Antimicrobial peptides	Enhanced resistance	_	Ghag et al. (2014)
Ced-9	Synthetic	*Fusarium oxysporum* race 1	RNAi	Enhanced resistance	_	Magambo et al. (2016)
Rice chitinase or ThEn-42 grape stilbene synthase	Rice, trichoderma, and grape	*Mycosphaerella fijiensis*	Inhibitory effect	Enhanced resistance	_	Vishnevetsky et al. (2011), Kovács et al. (2013)
Rep	Viral DNA	*Banana bunchy top virus*	Inhibit virus replication	Enhanced resistance		Elayabalan et al. (2013)
ihpRNA-Rep or *ihpRNA-ProRep*	Viral DNA	*Banana bunchy top virus*	RNAi	Enhanced resistance	_	Shekhawat et al. (2012)
Cystine inhibitor (cystatin)	Rice	*Radopholus similis*	Antifeedant	Enhanced resistance	_	Atkinson et al. (2004)
Cystine inhibitor (cystatin)	Maize	*R. similis, Helicotylenchus multicinctus*	Antifeedant	Enhanced resistance	Enhanced resistance	Roderick et al. (2012), Tripathi, Babirye et al. (2015)
Peptide	Synthetic	*R. similis, Helicotylenchus multicinctus*	Repellent	Enhanced resistance	Enhanced resistance	Roderick et al. (2012), Tripathi, Babirye et al. (2015)

TABLE 2 List of some of the recent advances in genome editing of crops for disease resistance

Crop	Editing system	Type of modification	Target gene	Target trait	Result	Reference
Apple	CRISPR/Cas9	Gene disruption	DIPM1, DIPM2, DIPM4	Fire blight disease	Mutations in target genes; disease resistance not checked	Malnoy et al. (2016)
Arabidopsis	CRISPR/Cas9	Gene disruption	eIF4E	*Turnip mosaic virus* (TuMV)	Complete resistance to TuMV	Pyott et al. (2016)
Banana	CRISPR/Cas9	Viral genome disruption	Viral genes	*Banana streak virus* (BSV)	Inactivation of endogenous banana streak virus integrated in host genome	Tripathi et al. (2019)
Cacao	CRISPR/Cas9	Gene disruption	*NPR3*	*Phytophthora tropicalis*	Resistance to fungal disease	Fister et al. (2018)
Cassava	CRISPR/Cas9	Gene disruption	*eIF4E isoforms nCBP-1 and nCBP-2*	*Cassava brown streak virus*	Resistance to brown streak disease	Gomez et al. (2019)
Citrus	CRISPR/Cas9	Gene disruption	CsLOB1	*X. citri* subsp. *citri*	Resistance to citrus canker	Jia et al. (2017)
Citrus	CRISPR/Cas9	Promoter disruption	CsLOB1 promoter	*X. citri* subsp. *citri*	Enhance resistance to citrus canker	Peng et al. (2017)
Cotton	CRISPR/Cas9	Gene disruption	*GhMYB25*	Verticillium wilt	Resistance to *Verticillium dahliae*	Li et al. (2017)
Cotton	CRISPR/Cas9	Gene disruption	*Gh14-3-3d*	Verticillium wilt	Resistance to *Verticillium dahliae*	Zhang et al. (2018)
Cucumber	CRISPR/Cas9	Gene disruption	eIF4E	CVYV, ZYMV, PRSV-W	Resistance to cucumber vein yellowing virus (CVYV), zucchini yellow mosaic virus (ZYMV), and papaya ringspot virus-type W (PRSV-W)	Chandrasekaran et al. (2016)
Grapes	CRISPR/Cas9	Gene disruption	MLO7	Powdery mildew	Mutations in target genes; disease resistance not checked	Malnoy et al. (2016)
Grapevine	CRISPR/Cas9	Gene disruption	*VvWRKY52*	*Botrytis cinerea*	Resistance to gray mold disease	Wang et al. (2018)
Rice	TALEN	Promoter disruption	*OsSWEET14*	*X. oryzae* pv. *oryzae*	Resistance to bacterial blight	Li et al. (2012)
Rice	CRISPR/Cas9	Promoter disruption	*OsSWEET11, OsSWEET14*	*X. oryzae* pv. *oryzae*	Mutations in promoter, resistance not checked	Jiang et al. (2013)
Rice	CRISPR/Cas9	Gene disruption	*OsSWEET14*	*X. oryzae* pv. *oryzae*	Resistance to bacterial blight	Zhou et al. (2015)
Rice	CRISPR/Cas9	Gene disruption	OsERF922	*Magnaporthe oryzae*	Resistance to rice blast	Wang et al. (2016)
Rice	CRISPR/Cas9	Gene disruption	*OsMPK5*	Fungal and bacterial pathogens	Mutations in target, resistance not checked	Xie and Yang (2013)
Rice	CRISPR/Cas9	Gene disruption	*eIF4G*	*Rice tungro spherical virus*	Resistance to rice tungro spherical disease	Macovei et al. (2018)
Tobacco	CRISPR/Cas9	Gene disruption	Three viral regions *(R, CP,* and *RCR)*	*Tomato yellow mosaic virus*	Significant reduction or attenuation of disease symptoms	Ali et al. (2015)
Tobacco	CRISPR/Cas9	Viral gene disruption	*43* regions in the viral genome	*Beet severe curly top virus*	Reduction in viral accumulation	Ji et al. (2015)

(Continues)

TABLE 2 (Continued)

Crop	Editing system	Type of modification	Target gene	Target trait	Result	Reference
Tobacco	CRISPR/Cas9	Viral genome disruption	*6* regions in the viral genome	*Bean yellow dwarf virus*	Reduction in viral accumulation	Baltes, Gil-Humanes, Cermak, Atkins, and Voytas (2014)
Tomato	CRISPR/Cas9	Gene disruption	*SlDMR6*	Bacterial diseases	Resistance to *Pseudomonas syringae*, *Phytophthora capsici*, and *Xanthomonas* spp.	de Toledo Thomazella et al. (2016)
Tomato	CRISPR/Cas9	Gene disruption	*SlMlo1*	Powdery mildew	Resistance to *Blumeria graminis* f.sp. *tritici*	Nekrasov et al. (2017)
Tomato	CRISPR/Cas9	Gene disruption	*SlJAZ2*	*P. syringae* pv. *tomato*	Resistant to the bacterial speck disease	Ortigosa et al. (2019)
Wheat	TALEN and CRISPR/Cas9	Gene disruption	*TaMLO-A1, TaMLO-B1, TaMLO-D1*	Powdery mildew	Enhanced resistance to *Blumeria graminis* f.sp. *tritici*	Wang et al. (2014)
Wheat	CRISPR/Cas9	Gene disruption	*TaEDR1*	Powdery mildew	Resistance to *Blumeria graminis* f.sp. *tritici*	Zhang et al. (2017)

2009). The disease affects production of all types of banana including EAHB, plantains, dessert, and juice-producing varieties (Ssekiwoko, Taligoola, & Tushemereirwe, 2006). The impacts of BXW are both severe and fast, unlike those of other diseases which gradually increase losses over years. The economic impact of BXW is because of the complete loss of yields and death of the mother plant that would otherwise contribute to the sucker plant production cycles (Tripathi et al., 2009). Overall, economic losses from BXW were estimated at US$ 2–8 billion over a decade, arising from price increases and significant reduction in production (Abele & Pillay, 2007).

The bacterial pathogen, Xcm, is mainly transmitted through insect vectors, infected planting material, and contaminated farming tools (Tripathi et al., 2009). Currently, the insect-mediated transmission is rare at higher altitudes (>1,600 masl) because of the lower insect population at low temperature, which is co-related with fewer incidents of BXW disease in those regions. With an increase in temperature, the insect population might also bloom in the regions of high altitude, causing greater impact of BXW disease on banana production.

There is no known source of resistance to BXW within the *Musa* except for wild-type diploid banana "*Musa balbisiana*" (Ssekiwoko et al., 2006). Current control relies upon improved phytosanitary practices and cultural control measures to reduce losses and limit the spread of the disease. Recently, transgenic banana expressing hypersensitive response-assisting protein (*Hrap*) and plant ferredoxin-like protein (*Pflp*) genes from sweet pepper (*Capsicum annuum*) was developed (Namukwaya et al., 2012; Tripathi et al., 2010). These transgenic bananas demonstrated a high level of resistance to BXW disease under field conditions in Uganda (Tripathi, Tripathi et al., 2014). The *Pflp* and *Hrap* genes enhance the hypersensitive response (HR) upon pathogen attack and have shown to provide resistance to various bacterial pathogens such as *Erwinia*, *Pseudomonas*, *Ralstonia,* and *Xanthomonas* in plants including tobacco, tomato, broccoli, rice, orchids, and potato (Huang et al., 2004; Liau et al., 2003; Tang et al., 2001). As single gene-based resistance can breakdown easily, transgenic bananas were also developed by pyramiding *Hrap* and *Pflp* genes (Muwonge, Tripathi, Kunert, & Tripathi, 2016). These transgenic plants need to be tested under different agroecological regions with different temperature gradients and relative humidity for trait durability under climate change.

We have also tested additional resistance genes, which can be used for gene stacking or pyramiding strategies in order to develop enhanced and durable resistance against BXW disease. The constitutive expression of the rice pattern recognition receptor (PRR), *Xa21,* in transgenic banana resulted in enhanced resistance to BXW disease (Tripathi, Lorenzen, Bahar, Ronald, & Tripathi, 2014).

Fusarium wilt is the most damaging fungal disease endangering banana production globally (Jones, 2000; Ploetz, 2015). It is responsible for severe infection, resulting in the complete wilting of plants and destruction of whole banana field. In 19th century, *Fusarium oxysporum* f. sp. *cubense* (Foc) race 1 wiped out "Gros Michel", the most commonly grown commercial banana variety at that time (Ploetz, 2015). "Gros Michel" was replaced by "Cavendish Williams", which is resistant to race 1. But now, the Foc tropical race 4 (TR4) is spreading fast and threatening the production of Cavendish as well as other varieties resistant to race 1 (Dale et al., 2017).

In the past, various transgenes have been used to develop genetically engineered banana and many conferred significant levels of resistance to fungal pathogens. Transgenic banana with rice thaumatin-like protein (TLP) or PR-5 gene has shown significant resistance against Foc race 1 (Mahdavi, Sariah, & Maziah, 2012). Resistance against Foc race 1 has also been demonstrated in transgenic banana using either the anti-apoptosis-related gene or through RNAi silencing of vital genes of Foc (Ghag, Shekhawat, & Ganapathi, 2014; Magambo et al., 2016; Paul et al., 2011). Recently, overexpression of *RGA2* or *Ced9* gene in transgenic Cavendish banana showed significant resistance against Foc under field conditions in Australia (Dale et al., 2017).

Black Sigatoka is another important fungal disease affecting banana production worldwide. Commercial growers apply fungicides to control the black Sigatoka infection, but smallholder farmers in Africa cannot afford to do this. Transgenic banana expressing rice chitinase or endochitinase gene (*ThEn-42*) from *Trichoderma harzianum* stacked with grape *stilbene synthase* (*StSy*) has shown resistance to this disease (Kovács et al., 2013; Vishnevetsky et al., 2011).

So far, no work has been published on the use of genome editing in banana for resistance to bacterial and fungal diseases. However, some advances on other crops have been reported for the development of different levels of immunity to biotic stresses using genome editing (Table 2). Susceptibility genes (S-genes) have been successfully edited to confer resistance to pathogens. For example, simultaneous mutations in three homeoalleles of mildew resistance locus O (MLO), *TaMLO-A1*, *TaMLO-B1,* and *TaMLO-D1,* in bread wheat by TALEN and CRISPR/Cas9 technologies, were shown to confer resistance to powdery mildew disease (Wang et al., 2014). Similarly, mutations in *SlMLO1* gene in tomato and *TaEDR1* in wheat enhanced resistance to powdery mildew disease (Nekrasov et al., 2017; Zhang et al., 2017). Mutation of the susceptibility gene for citrus canker, *LATERAL ORGAN BOUNDARIES* (*CsLOB1*), and promoter of *CsLOB1* by CRISPR/Cas9 conferred high degree of resistance against citrus canker caused by *Xanthomonas citri* subsp. *citri* (Jia et al., 2017; Peng et al., 2017). Downy mildew resistance 6 (*DMR6*), which belongs to the superfamily of 2-oxoglutarate Fe (II), is specifically upregulated during pathogen infections. Mutation of tomato DMR6 (*SlDMR6-1*) gene by CRISPR/Cas9 produced plants, which showed disease resistance against different pathogens, including *Pseudomonas syringae*, *Phytophthora capsici*, and *Xanthomonas spp*. (de Toledo Thomazella, Brail, Dahlbeck, & Staskawicz, 2016).

Genes in the metabolic pathway that regulate hormonal balance, sugar transport, and stomata opening have also been edited to confer plant immunity. Ethylene-responsive factor (ERF) in the ethylene pathway belongs to the transcription factor family APETALA2/ERF. ERF regulates molecular response to pathogen attack by binding to sequences containing AGCCGCC motifs (the GCC box), a cis-acting element. Using CRISPR/Cas9, the rice ethylene-responsive (*OsERF922*) gene was edited and the resulting plants conferred increased resistance to *Magnaporthe oryzae* (Wang et al., 2016). The disruption of the gene involved in sugar transport, *OsSWEET14*, through mutation in the promoter conferred enhanced resistance to bacterial blight disease in rice (Li, Liu, Spalding, Weeks, & Yang, 2012). Similarly, rice mutants generated using CRISPR/Cas9 tool to knockout *OsSWEET13* in rice showed enhanced resistance against *Xanthomonas oryzae* pv. *oryzae*, causing bacterial blight (Zhou et al., 2015). Tomato with regulated opening of stomata through the editing of *SlJAZ2* showed resistance against *Pseudomonas syringae* pv. *tomato* (*Pto*) causing bacterial speck disease (Ortigosa, Gimenez-Ibanez, Leonhardt, & Solano, 2019).

Genome editing of transcription factors such as MYB and WRKY showed protection against pathogens. Cotton plants with mutations in *GhMYB25* and *Gh14-3-3d* genes showed resistance to the fungal disease Verticillium wilt (Li, Unver, & Zhang, 2017; Zhang et al., 2018). Similarly, genome-edited grapevine with mutations in the *VvWRKY52* transcription factor demonstrated enhanced resistance to *Botrytis cinerea* (Wang et al., 2018). Recently, disease resistance to *Phytophthora tropicalis* has been demonstrated in cacao through CRISPR/Cas9 based editing of the *nonexpressor of pathogenesis-related 3* (*NPR3*) gene, a suppressor of the immune system (Fister, Landherr, Maximova, & Guiltinan, 2018).

Banana genomes can be edited in a way similar to other crops in a targeted manner to produce new varieties with increased tolerance to biotic stress. Similar strategies of editing the susceptible genes such as *MLO* and *DMR6* or their promoters can be applied in banana for developing resistance to bacterial and fungal diseases. Also, other components of immunity pathways such as pathogen recognition receptors, sugar transporters such as SWEET gene family, or transcription factors such as ERF, WRKY, and MYB genes can be mutated in banana to enhance resistance against bacterial and fungal pathogens.

Banana bunchy top disease (BBTD) is another major biotic stress globally widespread (Stainton et al., 2015). BBTV

is transmitted by aphids (*Pentalonia nigronervosa*), and aphids have been reported to spread virus much more efficiently at higher temperature (Anhalt & Almeida, 2008). Resistance against BBTV has been developed in banana using RNAi to silence the essential viral genes (Elayabalan et al., 2013; Shekhawat, Ganapathi, & Hadapad, 2012). RNAi does not lead every time to the complete silencing of genes; hence, CRISPR/Cas9 can be used to knockout the target genes. Genome editing, specifically CRISPR/Cas9, has been applied to introduce resistance to geminivirus, circular single-stranded DNA (ssDNA) viruses that replicate within the nuclei of plant cells, causing serious damage to many dicotyledonous crop. This system has been applied to develop resistance to *tomato yellow leaf curl virus* (TYLCV) in *Nicotiana benthamiana* (Ali et al., 2015), and *beet severe curly top virus* in *Arabidopsis thaliana* and *N. benthamiana* (Ji, Zhang, Zhang, Wang, & Gao, 2015). The eukaryotic translation initiation factor (*eIF*) gene family, including *eIF4E* and its paralogue *eIF(iso)4E*, which have been identified as recessive resistance alleles against various potyviruses, has also been edited to confer resistance to several viruses. CRISPR/Cas9 genome editing of *eIF(iso)4E* gene in cucumber showed resistance to *cucumber vein yellowing virus* (CVYV), *zucchini yellow mosaic virus* (ZYMV), and *papaya ringspot virus*-type W (PRSV-W) (Chandrasekaran et al., 2016). Using a similar approach, Pyott, Sheehan, and Molnar (2016) showed that mutation of *eIF(iso)4E* locus in Arabidopsis successfully engineered complete resistance to *turnip mosaic virus* (TuMV). The editing of *eIF* gene family in banana can provide resistance to BBTV, which is an ssDNA babuvirus.

CRISPR/Cas9 technology was also applied in plants for developing resistance to single-stranded RNA (ssRNA) viruses such as *rice tungro spherical virus* and *cassava brown streak virus*. Disease resistance has been developed against *rice tungro spherical virus* by editing of *eIF4G* alleles in rice plants (Macovei et al., 2018). Recently, it has been reported that cassava plants with editing of *eIF4E* isoforms *nCBP-1* and *nCBP-2* demonstrated a reduction in symptom severity and incidence of cassava brown streak disease (Gomez et al., 2019).

Banana streak virus is also one of the major constraints for banana production. It is dsDNA virus belonging to badnaviruses, which integrates in the host plant genome (Harper, Osuji, Heslop-Harrison, & Hull, 1999). Under the stress conditions such as temperature, drought, crossing, and micropropagation, the integrated viral sequences get activated and produce the infectious episomal form of BSV and plants develop symptoms. Climate change particularly extreme temperature and drought can make BSV worse in plantations. CRISPR/Cas9 system has been applied to knockout the integrated virus sequences from the host genome eliminating the chances of their activation to infectious viral particles (Tripathi et al., 2019).

Nematodes and weevils are serious pests of banana causing losses up to 40%–50% (Gold, Pena, & Karamura, 2001; Jones, 2009). Several nematode species such as *Radopholus similis*, *Pratylenchus goodeyi*, *P. coffeae*, *Helicotylenchus multicinctus,* and *Meloidogyne* spp. are present either alone or in combination in banana plantations (Coyne, Omowumi, Rotifa, & Afolami, 2013). Commercial farmers apply pesticides to control nematodes, but this is environmentally unsafe and not affordable for smallholder farmers. Transgenic banana cultivar "Cavendish Williams" harboring rice cystatin showed resistance against *R. similis* under glasshouse conditions (Atkinson, Grimwood, Johnston, & Green, 2004). Later, transgenic plantain cultivar "Gonja Manjaya" expressing antifeedant cysteine proteinase inhibitor from maize and an antiroot invasion peptide either singly or stacked demonstrated a high level of resistance against *R. similis* and *H. multicinctus* under glasshouse and field conditions (Roderick et al., 2012; Tripathi, Babirye et al., 2015). These transgenic plantains can also provide resistance to other species of nematodes for instance *P. goodeyi*, *P. coffeae*, and *Meloidogyne* spp.

Cysteine proteinase inhibitors can also provide resistance to banana weevils (*Cosmopolites sordidus*). Kiggundu et al. (2010) have demonstrated the deleterious effect of cysteine proteinases from rice and papaya on banana weevils.

So far, there are no reports of the genome editing of crops for developing resistance to nematodes and weevils. Genome editing has been established in the free-living nematode, *Caenorhabditis elegans,* which will allow the identification of essential genes involved in the different physiological processes of nematodes (Dickinson & Goldstein, 2016; Paix, Folkmann, & Seydoux, 2017).

Extreme temperature could affect plant–pathogen interactions and disease resistance. It is reported that the resistance (R) gene-mediated disease resistance against bacterial and viral pathogens has been reported to be suppressed by a rise in temperature (Wang, Bao, Zhu, & Hua, 2009). The banana and plantain varieties developed for diseases and pest resistance should be tested for durability of the traits under different temperatures and should be coupled with tolerance to abiotic stresses such as drought and high temperature.

7 | DROUGHT AND TEMPERATURE TOLERANCE

The most serious abiotic stresses for banana production are drought and heat due to irregular rainfall and increase in temperature. Annual rainfall of 2,000–2,500 mm all year is necessary for banana growth. Van Asten et al. (2010) estimated yield losses of about 65% from drought stress in rain-fed banana production. The majority of the banana varieties with AAA are susceptible to drought, but varieties with AAB or ABB genome constitution are drought-tolerant

(Vanhove, Vermaelen, Panis, & Swennen, 2012). The comparative transcriptome profile of drought-sensitive banana cultivar "Grand Naine" (AAA) and drought-tolerant banana cultivar "Saba" (ABB) under drought stress and control condition identified several differentially expressed genes associated with drought tolerance (Muthusamy, Uma, Backiyarani, Saraswathi, & Chandrasekar, 2016). This information can be used for developing drought-tolerant varieties of banana.

Information available from other crop species can also be transferred to banana using genetic engineering. Drought-tolerant maize was developed by expressing MAPK gene, which induces the oxidative signaling pathway (Shou, Bordallo, & Wang, 2004). Overexpression of WRKY transcription factor from rice (*OsWRKY11*) and barley (*Hv-WRKY38*) demonstrated their role in conferring tolerance to drought and extreme temperatures (Mare et al., 2004; Wu, Shiroto, & Kishitani, 2009). Similarly, co-expression of bZIP transcription factor (*OsbZIP46CA1*) along with protein kinase (*SAPK6*) involved in the ABA signaling pathway showed improved tolerance to heat and cold stresses in rice (Chang et al., 2017). Also, transgenic rice overexpressing C_4 phosphoenolpyruvate carboxylase (PEC) photosynthesis enzymes conferred drought tolerance (Gu, Qiu, & Yang, 2013). Furthermore, Yu et al. (2017) have demonstrated that overexpression of poplar genes *PtPYRL1* and *PtPYRL5* enhances tolerance to drought and cold stresses by activating the ABA signaling pathway. The cap-binding protein 80 (CBP80, also known as *abscisic acid hypersensitive 1* gene) in *A. thaliana* has been reported to play an important role in drought tolerance. The inactivation of CBP80 in *A. thaliana* resulted in ABA-hypersensitive stomatal closing and reduced wilting during drought (Hugouvieux, Kwak, & Schroeder, 2001; Kmieciak, Simpson, Lewandowska, Brown, & Jarmolowski, 2002). Loss of function of the Arabidopsis CBP20 resulted in hypersensitivity to ABA during germination and increased tolerance to water deficiency during drought stress (Papp, Mur, Dalmadi, Dulai, & Koncz, 2004). Later, silencing of CBP80 in potato showed enhanced tolerance to drought (Pieczynski et al., 2013).

Manipulating transcription factors or signaling pathways associated with abiotic stresses can generate drought-tolerant varieties of banana. Genome editing can be a valuable weapon in generating abiotic stress tolerance in crops, although not so much has been done in this area. So far, the only work using CRISPR/Cas9 genome editing to confer abiotic stress tolerance was demonstrated in maize and wheat (Kim, Alptekin, & Budak, 2018; Shi et al., 2017). The maize lines carrying *ARGOS8* variants showed tolerance to drought and increased yield (Shi et al., 2017). Recently, wheat has been edited for abiotic stress tolerance using CRISPR/Cas9 system targeting dehydration-responsive element-binding protein 2 (*TaDREB2*) and ethylene-responsive factor 3 (*TaERF3*) (Kim et al., 2018).

8 | CONCLUSIONS AND PERSPECTIVES

Climate changes are predicted to have effects on banana production similar to those in other crops. Modern breeding tools especially genetic modification and genome editing can be used in complementation with conventional breeding for developing climate-smart varieties of banana. Availability of banana genome sequences and the robust genetic transformation system allows researchers to apply genetic modification and genome editing for improvement of banana. In comparison with genetic modification using foreign genes, gene editing is more precise and cost-effective. Improved varieties developed through genetic modification require regulatory approval; however, gene-edited products having only a simple deletion, base pair swap, or insertion from reproductively compatible relatives are not regulated in several countries. The ability to generate transgene-free gene-edited varieties provides new opportunities for crop improvement.

Genome editing can also be applied for functional genomics allowing identification of genes associated with climate-related agronomic traits, and these could be used for the improvement of banana in adaptation to the changing climate. As there are predictions that climate change, particularly a rise in temperature, will increase chances of the emergence of highly aggressive and invasive strains of pathogens and also bloom population of pests, the development of improved banana varieties is critical to deal with new strains of pathogens and increased populations of pests.

In order to broaden the availability of the gene pool for the improvement of banana, genes from wild-type progenitors of banana may need to be complemented with the use of novel transgenes from other species. Wild-type banana progenitor *Musa balbisiana* is known to have resistance to several diseases such as BXW and black Sigatoka, but breeders do not use this in their breeding programs due to issue of integrated endogenous BSV sequences. With the availability of the genomic sequence of *M. balbisiana*, new breeding tools such as genome editing can use the information to improve cultivated banana for disease resistance.

Extreme weather might suppress the immunity of banana against pathogens; therefore, evaluation of the available disease-resistant varieties of banana under conditions of higher temperature is needed to identify sustainable resistance allowing plants to cope with epidemics under elevated temperature. Improved banana varieties need to be developed which can resist combinations of abiotic–biotic stresses.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

LT is responsible for the original concept. VN and JT contributed in writing and making figures and editing of the manuscript.

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