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Prospects and Challenges of Genome Editing For Disease Resistance in Banana (*Musa* Spp.)

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ARTICLE ID: 042

Abstract

Viral diseases are significant biological restrictions on the production of bananas (*Musa* spp.), because they affect yields. Of all the viruses known to infect bananas, the banana bunchy top virus and the banana streak virus are widespread and economically harmful. The use of virus-resistant bananas is the most cost-effective option for minimizing the adverse effects of viral diseases on banana production. CRISPR / Cas-based genome editing has proven to be the most powerful tool for developing virus-resistant plant varieties in several crops, including bananas. Powerful genetic transformation and regeneration systems and the availability of well-annotated whole-genome sequences from bananas make them attractive candidates for genome editing. Robust CRISPR / Cas9-based genome editing for bananas has recently been established and can be used to develop disease-resistant varieties. Recently, the CRISPR system has been used to detect target gene sequences with the enzymes Cas9, Cas12, Cas13, and Cas14, revealing the use of this technique for viral diagnosis. This article outlines recent advances and prospects in diagnosing and developing resistance to banana diseases using CRISPR / Cas-based genome editing, as well as challenges in banana genome editing.

Keywords: Genome editing, banana, disease, resistance

Introduction

Banana (*Musa* spp.) is one of the most important staple foods. In addition to contributing to food security as a staple food, it also generates income as a cash crop, especially in tropical and subtropical countries. The global banana value chain is increasingly shaped by the downstream activities of large retail chains from the most important importing countries (Figure 1). These chains operate independently of traditional fruit companies by procuring bananas directly from producers and dealers. Several devastating phytopathogens

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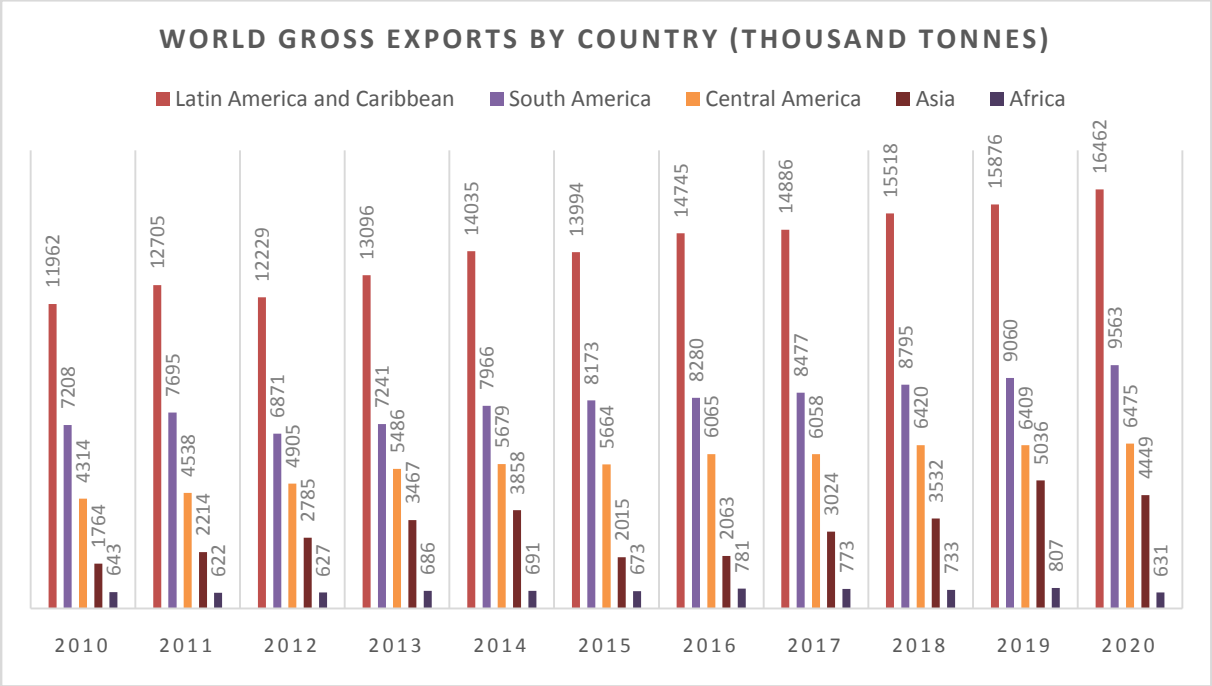
attack bananas. The biggest challenge facing agriculture is to feed a growing population, which is projected to reach 9.7 billion in 2050 and 10.8 billion in 2100, compared to 7.7 billion in 2019. To meet growing food demand with limited resources, it is needed better and more efficient ways to produce food. The development of disease-resistant bananas by traditional breeding is a major challenge due to interspecific crossing barriers that impede the transfer of desirable agricultural traits to the genus.

The main problems with traditional breeding are ploidy, long production cycles, sterility in most varieties, and low genetic diversity of *Musa* sp. In addition, the introduction of some fungal, bacterial, and viral resistance genes into crops can significantly reduce yields and enhance other agriculturally undesirable traits due to genetic associations. Biotechnology tools such as genetic modification (GM) and genome editing (GE) provide low-cost strategies for developing improved banana varieties that are resistant to multiple diseases. Currently, serious efforts are being made to develop GM banana varieties that are resistant to diseases. The commercialization of GM plants faces hurdles due to complex regulatory approval procedures. Genome editing approaches have the ability to accelerate breeding by making efficient and accurate changes to the plant genome to develop new traits such as disease resistance. This article describes how to use genome editing to develop disease-resistant bananas.



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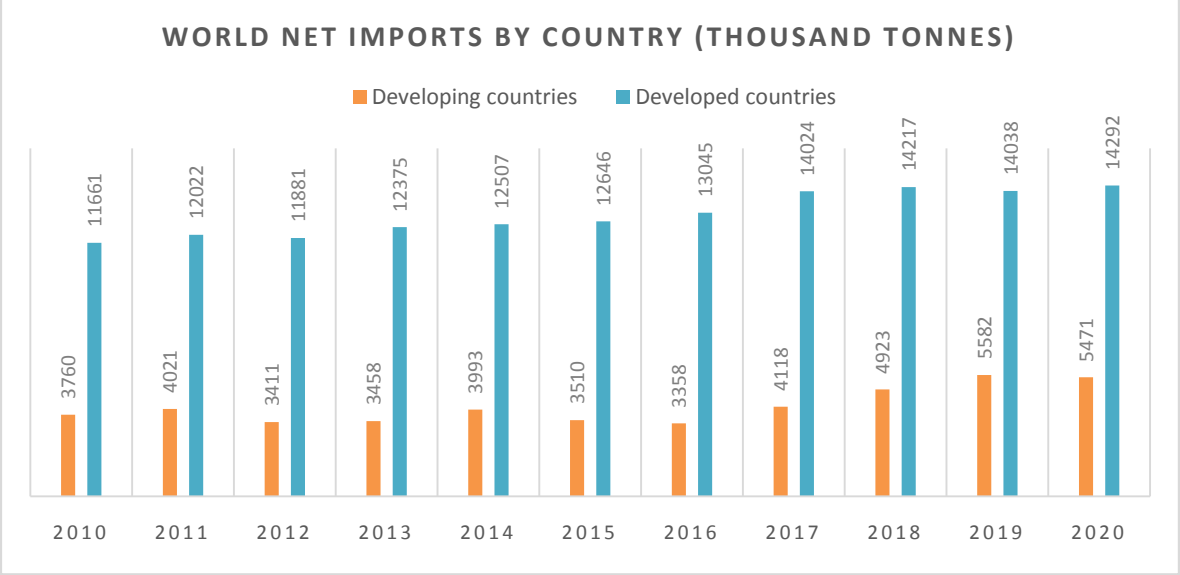

Figure 1. Banana Statistics (Source: Banana Statistical Compendium 2020 – FAO)

Some important varieties of banana

Edible banana varieties are obtained from interspecific crosses: *Musa acuminata* X *Musa bulbisiana*. Ripe fruits are a good source of vitamin A, a good source of vitamins C, B1 and B2, and contain up to 27% sugar. Unripe banana berries and the inner core of the pseudo-stem are regularly cooked as curry. Banana leaves are considered biological plates. Some important banana varieties are shown in Table 1 and Figure 2. Each specification may be subjected to disease resistance in genome editing.

Table 1. Some important varieties of banana

Variety name	**Specification**
Poovan	Also known as Champa or Larbergi. It is resistant to Panama wilt disease and is strongly affected by the banana streak virus.
Monthan	It is called Bonta, Canchikera, Pontan. It is resistant to drought and is cultivated for leaf production.
Dwarf cavendish	It is called Basrai, Kadari, Pachabazai. It accounts for 58% of production and is a major commercial strain resistant to Panama wilt disease.
Harichal	Locally known as Robusta, Bombay Green. It is an intermediate of Dwarf Cavendish.
Rasthali	Locally known as Maltaman. It's the most exquisite table type, but the

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	problem is that it forms hard lumps and cracks in the fruit.
Hill banana	Syn. Virupakshi, Sirumalai, Vellavazhai. It is suitable for higher elevation.
Grand Naine	Imported from France. A large variant of Drwarf Cavendish. Today, it is of increasing commercial importance due to its high yield and high quality.
Nendran	It is having good keeping quality.
Karpuravalli	Withstands drought, salt and wind, suitable for juice and wine production

Figure 2. Important varieties of banana; can be targeted for genome editing for disease resistance

Important diseases in banana

Banana production is severely constrained by several diseases and pests, particularly in regions where various pests and pathogens co-exist. Prominent among these diseases are banana *Xanthomonas* wilt caused by *Xanthomonas campestris* pv. musacearum, black Sigatoka caused by *Pseudocercospora fijiensis*, *Fusarium* wilt, commonly known as Panama disease, caused by *Fusarium oxysporum* f. sp. cubense (Foc), banana bunchy top disease, and banana streak disease and pests such as nematodes and weevils. Banana *Xanthomonas* wilt

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disease is considered one of the most significant production constraints for the banana in Central and East Africa. Severe risks to global production of banana are currently posed by *Fusarium oxysporum* f. sp. cubense tropical race 4 (TR4). The emergence of this new threat to banana production has created an urgency to develop disease-resistant varieties using new breeding tools such as GE.

Prospects of genome editing for disease resistance in banana

The application of new breeding methods for agricultural productivity is a central concern to achieve global food security. By manipulating plant genes of interest in crops using various site-specific nucleases (SDNs) such as zinc finger nucleases (ZFNs), meganucleases (MNs), and transcriptional activator-like effector nucleases (TALENs). The CRISPR / Cas9 system has proven to be the most powerful tool for targeted GEs, including gene knockout, base exchange, multiple gene editing, and regulation of gene transcription in plants. The CRISPR / Cas9 tool is based on the induction of double-strand breaks (DSBs) at the target site and the repair of breaks by either homologous-directed repair (HDR) or non-homologous ends (NHEJ). It creates user-requested mutations, from target point mutations to large deletions or insertions of exogenous DNA at target sites in the genome.

There are four different types of processing. SDN1, SDN2, SDN3 and basic processing. SDN1 is a highly efficient and error-prone repair of the target DSB by NHEJ, causing gene silencing, gene knockout, or mutations that cause altered gene function. SDN2 is a template-driven repair of the target DSB using a repair template with one or more small mutations in which two sequences matching both ends of the DSB are adjacent, with low efficiency, high fidelity, generated by HDR. It will be configured. This type of repair allows mutations to be introduced into the target site. SDN3 is inefficient, is produced with high fidelity by HDR, and uses the donor sequence to insert the entire gene or gene element into the target site through template-guided repair of the target DSB. SDN1 and SDN2 are similar to mutations obtained by chemical mutagenesis, radiation, or spontaneous spontaneous mutation. Base editing results in accurate single nucleotide changes in genomic DNA or cellular RNA, independent of the need for DSBs, DNA donor templates, or HDR. Editing bases does not require a DNA donor template and can be considered SDN1. This is an important development as bananas are polyploid and difficult to improve with traditional

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breeding approaches. Genome editing technology has been successfully studied for the development of disease-resistant banana varieties (Table 2).

Table 2. Genes available for genome editing of banana for disease resistance

Target gene	**Trait**	**Reference**
MLO13, DMR6; SWEET14; E3 ubiquitin ligases	*Xanthomonas* wilt	López-Calleja *et al.*, 2019
eIF, Rep, ihpRNA-Rep or *ihpRNA-ProRep*	Banana bunchy top virus	Tripathi *et al.*, 2019
ORF1, ORF2, and ORF3 of BSV	Banana streak virus	Tripathi *et al.*, 2019
RGA2 or Ced9	*Fusarium oxysporumcubense* tropical race 4	Tripathi *et al.*, 2019
TLP or PR-5, Antiapoptosis genes *(Bcl-xL, Ced-9, Bcl-2), PhDef1 and PhDef2, Ced-9*	*Fusarium oxysporumcubense* race 1	Tripathi *et al.*, 2019
Hrap, Pflp, Xa21	*X. campestris* pv.*musacearum*	Tripathi *et al.*, 2019

Challenges of genome-editing inbanana

Bananas are polyploid heterozygous crops and contain a large number of multigene families with paralogs. One of the major challenges in banana genome editing is the simultaneous targeting of multiple alleles and gene copies. Knockdowns or knockouts of certain genes may not result in phenotypic changes, probably due to the dose effect of other paralogous gene copies. Therefore, gRNAs should be designed to target all copies of the gene and alleles, screen many variants, and obtain edited multi-allelic lineages. Multiple genome editing using multiple gRNAs targeting multiple genes can be an efficient tool for addressing polyploid crops (Ansari *et al.*, 2020).

Another major challenge is that genome editing of banana crops is now achieved by plasmid-based introduction by transformation of Agrobacterium (Ntui *et al.*, 2020). However, temporary delivery systems such as agroinfiltration and protoplast fusion are not successful with bananas. Mutants generated by stable transformation are considered GMOs due to the integration of transgenes into the plant genome and require time-consuming regulatory approval, which may reduce their acceptance. Because bananas are vegetative crops, the

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separation of Cas9 genes, marker genes, and Agrobacterium-derived DNA sequences by backcrossing, in contrast to sown plants, is absent from the majority of cultivars preferred by farmers (Nadakuduti *et al*., 2018; Tripathi *et a*l., 2020a; Tripathi *et al.*, 2020b).

To address these biosafety concerns, it is necessary to develop genome-edited banana plants that do not contain transgenes. For bananas, pre-assembled RGENs RNPs that target viral genes or plant host factors can be coated with gold particles and delivered to banana cell suspension cultures by particle impact. Alternatively, a CRISPR / Cas9 construct that targets a viral gene or plant host factor can be temporarily introduced into banana cells by microprojection impact. The greatest biosafety concern in genome-edited plants is the undesired genetic alteration of the plant due to untargeted mutations and transgene integration (Tripathi et al., 2020b). Off-target effects can be minimized by making the gRNA design strategy highly specific for target and RNP-based delivery.

Another pressing issue is the lack of high-throughput screening methods to identify genome-edited events. Treated banana plants are currently being screened using PCR and target sequencing to identify mutations. However, these methods are expensive and time consuming. After screening the treated plants using the high-throughput phenotype of the trait of interest, targeting the selected event is more efficient and cost-effective (Tripathi *et al*., 2020a).

Conclusion

CRISPR-related protein-based genome editing is rapidly revolutionizing the use of crop fortification for desirable traits such as disease resistance. BBTV and BSV are economically important viruses that threaten banana production. The most sustainable way to reduce the loss from these viral diseases is to use virus-resistant banana varieties. In the absence of known resistant genetic resources, long-lived virus-resistant varieties should be developed using the latest biotechnology tools that complement traditional breeding. Genome editing tools provide a new weapon against plant viruses and other disease causing agents. The availability of a robust genome editing system and reference genome makes bananas a potential candidate for the development of disease-resistant varieties. So far, genome editing has only been used in bananas to control BSV by inactivating eBSV integrated into the host genome. A CRISPR / Cas system that targets the essential or host plant genes of the virus involved in susceptibility can be used in bananas to develop resistance to BBTV (Tripathi *et*

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al., 2020a). Genome-edited disease-resistant banana varieties are suitable for commercialization because they can be produced without incorporating foreign genes.

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