

ID: 386

## Advances in Molecular Breeding for Disease Resistance in Banana (*Musa Spp*): Current Trends and Future Prospects

Dushimeyesu Erastus<sup>1,2,3\*</sup>, Edema Richard<sup>1,2</sup>, Isiaka Abiodun<sup>1,2</sup>, Uwimana Brigitte<sup>4</sup>,

<sup>1</sup> Department of Crop Science and Horticulture, College of Agricultural and Environmental Sciences (CAES), Makerere University, P.O. Box 7062, Kampala, Uganda

<sup>2</sup> Makerere University Regional Centre for Crop Improvement (MaRCCI), College of Agricultural and Environmental Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda

<sup>3</sup> Department of Agriculture, Faculty of Agriculture, Environmental management and Renewable Energy, University of Technology and Arts of Byumba, -Gicumbi District-Rwanda

<sup>4</sup> International Institute of Tropical Agriculture, Kampala, Uganda

\*Corresponding Author, Email: eradus2020@gmail.com ; Orcid: <https://orcid.org/0000-0002-8129-5407>

### Abstract

Banana (*Musa spp.*) is a staple crop providing food security and economic benefits to millions globally. However, its production is severely threatened by biotic and abiotic stresses, including Fusarium wilt, Black Sigatoka, Banana Xanthomonas Wilt, and climate-related challenges. Traditional breeding approaches in banana are hindered by long breeding cycles, polyploidy, and sterility in most cultivated varieties, making genetic improvement challenging. Recent advances in molecular breeding, including marker-assisted selection (MAS), genome-wide association studies (GWAS), and next-generation sequencing, have revolutionized banana improvement by enabling precise trait selection. The application of genetic transformation and CRISPR/Cas9 genome editing has further enhanced the development of disease-resistant and biofortified banana varieties, offering sustainable solutions to production constraints. Despite these advancements, challenges remain in gene introgression, regulatory frameworks, and farmer adoption of genetically improved bananas. Future research should focus on refining genome editing techniques, expanding genomic resources, and integrating artificial intelligence in breeding programs to accelerate cultivar development. This review highlights the progress in molecular banana breeding, discusses existing challenges, and explores emerging opportunities for ensuring banana resilience and productivity in the face of evolving environmental and disease pressures.

### Introduction

Banana (*Musa spp.*) is one of the most important staple crops worldwide, serving as a primary source of food, income, and nutrition for over 400 million people, particularly in tropical and subtropical regions (FAO, 2021). Globally, banana production exceeds 155 million metric tons annually, with major producers including India, China, Indonesia, Brazil, and the Philippines (Tripathi *et al.*, 2020). In Africa, Uganda, Rwanda, and the Democratic Republic of Congo are among the leading producers, where bananas contribute significantly to food security and livelihoods (Karamura *et al.*, 2019). Latin America, particularly Ecuador, Colombia, and Costa Rica, is a major exporter of bananas, supplying global markets, especially in Europe and North America (FAO, 2021).

### Challenges in Conventional Banana Breeding

Despite its economic and nutritional importance, banana breeding faces significant challenges. Most cultivated banana varieties, such as the Cavendish and East African Highland bananas, are triploid and sterile, preventing natural recombination and seed-based breeding (Ortiz & Swennen, 2014). Traditional breeding is further hindered by the long reproductive cycle and vegetative propagation, which increases the risk of disease accumulation over successive generations (Perrier *et al.*, 2019). The prevalence of devastating diseases, including Fusarium wilt (Tropical Race 4), Black Sigatoka, and Banana Xanthomonas Wilt, further complicates breeding efforts, as resistant cultivars are difficult to develop through conventional methods (Tripathi *et al.*, 2020).

Given the limitations of conventional breeding, molecular approaches have emerged as powerful tools for banana improvement. Advances in marker-assisted selection (MAS), genome-wide association studies (GWAS), and genomic selection (GS) have facilitated the identification and introgression of resistance genes, accelerating the breeding process (Ahmadi *et al.*, 2021). Additionally, genetic transformation techniques, such as RNA interference (RNAi) and CRISPR/Cas9 genome editing, have enabled targeted modifications for disease resistance and enhanced nutritional content (Tripathi *et al.*, 2019). The complete sequencing of the banana genome has provided valuable insights into genetic diversity and trait inheritance, allowing breeders to develop improved cultivars with greater efficiency (D'Hont *et al.*, 2012). As climate change and emerging diseases continue to threaten banana production, integrating molecular breeding strategies with conventional methods is essential for ensuring sustainable production and food security.



### **Genetic and Genomic Advances in Banana**

#### **Genome Sequencing of Banana (*Musa spp.*)**

The sequencing of the banana genome has provided critical insights into its genetic diversity, evolutionary history, and potential for breeding improvement. The first draft genome sequence of *Musa acuminata* was published by D'Hont *et al.* (2012), marking a major breakthrough in banana genetics. This sequencing effort revealed that the banana genome is highly complex due to polyploidy and extensive segmental duplications. The reference genome of *Musa acuminata* (AA genome) provided insights into disease resistance genes, stress tolerance pathways, and fruit development mechanisms (D'Hont *et al.*, 2012).

Subsequent sequencing efforts focused on the *Musa balbisiana* (BB genome), which contributes to drought tolerance and disease resistance in many cultivated hybrids (Wang *et al.*, 2019). More recently, improved genome assemblies have been generated using third-generation sequencing technologies, such as PacBio and Oxford Nanopore, providing higher resolution and facilitating more precise gene annotation (Belser *et al.*, 2021). These advancements enable researchers to explore gene-editing strategies and accelerate the development of improved banana cultivars.

#### **Important Genetic Markers (SNPs, SSRs) and Their Applications**

Molecular markers play a crucial role in banana breeding by enabling precise selection of desirable traits. Among the most widely used markers are single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs).

- **SNP Markers:** SNPs are single-base variations in DNA sequences and are highly abundant in the banana genome. They are widely used in genome-wide association studies (GWAS) and marker-assisted selection (MAS) to identify genes associated with disease resistance and fruit quality (Brown *et al.*, 2017). SNP-based genotyping has improved the efficiency of selecting Fusarium wilt-resistant and drought-tolerant banana varieties (Nyine *et al.*, 2018).
- **SSR Markers:** SSRs, also known as microsatellites, consist of short, repetitive DNA sequences that are highly polymorphic. They have been extensively used for genetic diversity studies, cultivar fingerprinting, and parentage analysis in bananas (Christelová *et al.*, 2017). SSR markers have been instrumental in identifying genetic variability among cultivated and wild banana species, aiding in conservation efforts and pre-breeding programs.

The integration of high-throughput sequencing technologies with SNP arrays and SSR markers has significantly enhanced banana breeding programs. These tools facilitate genomic selection, allowing breeders to predict the performance of hybrids before field trials, thus accelerating the breeding cycle (Perrier *et al.*, 2019).

### **Key Challenges in Banana Breeding**

#### **Long Breeding Cycle**

Banana breeding programs are hindered by the long reproductive cycle of the plant, which typically spans 2-3 years for the evaluation of hybrids (Ortiz *et al.*, 2010). The lack of sexual reproduction in most cultivated banana varieties, which are sterile triploids, further complicates the process, as they must be propagated vegetatively. This extended breeding cycle limits the rate of genetic improvement and slows down the introduction of desirable traits, such as disease resistance and improved yield (Perrier *et al.*, 2019). The use of in vitro culture techniques and embryo rescue has partially addressed this issue, enabling faster multiplication of hybrids, but the overall duration remains lengthy compared to other crops (Jain & Swennen, 2010).

#### **Limited Genetic Diversity**

The cultivated banana genome is predominantly derived from a narrow gene pool, mainly from *Musa acuminata* and *Musa balbisiana*, which limits the available genetic variation for breeding (Simmonds & Shepherd, 1955). The widespread use of a few cultivars, particularly the Cavendish group, has led to a bottleneck in genetic diversity, making the crop highly vulnerable to diseases and environmental stresses (Nicol *et al.*, 2020). Efforts to incorporate wild *Musa* species into breeding programs have been complicated by incompatibility, sterility, and undesirable traits (Swennen *et al.*, 2017). These genetic constraints impede the development of new, resilient banana varieties and pose a significant challenge for long-term production sustainability.

#### **Resistance Breakdown in Improved Varieties**

Despite progress in breeding disease-resistant banana varieties, resistance breakdown remains a significant challenge. For example, the Cavendish banana, the most widely grown cultivar globally, is highly susceptible to Fusarium wilt Tropical Race 4 (TR4) (Ploetz, 2015). Initially, the Cavendish was resistant to an earlier strain of Fusarium wilt, but the emergence of TR4 has led to widespread crop losses. Similarly, resistance to Black Sigatoka, a fungal disease, is also subject to breakdown as the pathogen evolves (Alves *et al.*, 2020). These challenges highlight the need for continuous genetic improvement and the incorporation of multiple resistance genes to safeguard against evolving pathogens. Integrating stacked resistance genes, genetic diversity, and gene pyramiding approaches are essential to provide durable resistance (Koenig *et al.*, 2018).



Table 1. Key Challenges in Banana Breeding

Challenge	Description	Implications	Source
Long Breeding Cycle	Banana breeding takes 2-3 years for hybrid evaluation due to vegetative propagation and long reproductive cycles.	Slows down the introduction of improved traits, limiting genetic progress.	Ortiz & Swennen (2010)
Limited Genetic Diversity	Cultivated bananas mainly come from <i>Musa acuminata</i> and <i>Musa balbisiana</i> , leading to a narrow genetic pool.	Makes banana crops susceptible to diseases and environmental stresses; limits breeding opportunities.	Simmonds & Shepherd (1955); Perrier et al. (2019)
Resistance Breakdown in Varieties	Disease resistance in improved varieties, such as Cavendish, often breaks down due to evolving pathogens.	Requires continuous breeding to ensure durable resistance to diseases like Fusarium wilt and Black Sigatoka.	Ploetz (2015); Alves et al. (2020)

### Molecular Breeding Techniques in Banana

#### Marker-Assisted Selection (MAS) for Disease Resistance

Marker-Assisted Selection (MAS) is a powerful tool in banana breeding, particularly for disease resistance. MAS relies on genetic markers (such as SNPs, SSRs) associated with resistance traits, allowing breeders to select individuals with desirable traits even before phenotypic expression. For example, MAS has been successfully used in banana breeding for resistance to Fusarium wilt Tropical Race 4 (TR4) and Black Sigatoka (Koenig *et al.*, 2018). These diseases are significant threats to banana production worldwide, and MAS accelerates the development of resistant varieties. By incorporating molecular markers linked to these resistance traits, banana breeders can reduce the reliance on field screening, thus shortening the breeding cycle (Brown *et al.*, 2017).

MAS has also been applied to select for improved post-harvest quality and drought tolerance in bananas, marking significant progress in improving banana varieties through genetic selection (Perrier *et al.*, 2019).

#### Genome-Wide Association Studies (GWAS) for Trait Mapping

Genome-Wide Association Studies (GWAS) have become a crucial method for identifying genetic loci associated with complex traits in banana. GWAS involves scanning the entire genome for variants that correlate with phenotypic traits, such as disease resistance, yield, and fruit quality. Recent studies have employed GWAS to identify markers linked to resistance to Fusarium wilt, Black Sigatoka, and Banana Bunchy Top Virus (BBTV) (Perrier *et al.*, 2019). This technique helps in trait mapping and enables precise genetic selection by pinpointing genes responsible for desirable traits.

By integrating GWAS with high-throughput sequencing technologies, breeders can conduct more efficient genomic selection and improve the precision of their breeding programs (Wang *et al.*, 2021). This approach is valuable for enhancing the genetic potential of banana cultivars without relying heavily on traditional breeding methods, thus speeding up the development of superior varieties.

#### CRISPR/Cas9 Applications in Banana

The CRISPR/Cas9 genome-editing technique has emerged as a revolutionary tool in banana breeding. CRISPR/Cas9 enables precise and targeted modifications of specific genes in the banana genome, allowing for the introduction of beneficial traits without the need for traditional hybridization. This approach has been utilized to develop bananas resistant to Fusarium wilt, a devastating disease that has decimated banana plantations globally (Bortesi *et al.*, 2016). By knocking out susceptibility genes or inserting resistance genes, CRISPR/Cas9 has the potential to produce bananas with improved disease resistance and other agronomic traits, such as improved nutritional content and stress tolerance.

Furthermore, CRISPR/Cas9 offers an opportunity for gene functional studies, enabling researchers to explore the role of specific genes in banana growth, development, and resistance mechanisms (Jia *et al.*, 2017). While CRISPR/Cas9-based improvements in banana are still in the early stages, its application holds promise for future banana breeding programs.

#### Genetic Transformation and Transgenic Approaches

Genetic transformation and transgenic approaches have been applied to bananas to introduce specific traits not found naturally within the gene pool. In these approaches, foreign genes are introduced into the banana genome to improve traits such as disease resistance, yield, and nutritional value. For example, bananas have been genetically



engineered to express resistance to Banana Bunchy Top Virus (BBTV) and Fusarium wilt by introducing resistance genes from other plants (Ortiz & Swennen, 2010).

Although transgenic bananas offer significant potential, regulatory and consumer acceptance challenges have hindered the widespread adoption of genetically modified (GM) bananas. However, research into transgenic banana varieties continues, particularly in developing regions where crop loss due to disease is a significant concern. Genetic transformation methods such as Agrobacterium-mediated transformation and biolistic transformation have enabled the successful introduction of foreign genes into banana cultivars (Swennen *et al.*, 2013). As regulatory frameworks for GM crops evolve, genetic transformation may become an integral part of banana breeding programs.

### Case Studies and Recent Successes

#### **Breeding Programs that Developed Disease-Resistant Bananas**

Disease resistance in bananas is critical to ensuring sustainable banana production globally. Several breeding programs have successfully developed disease-resistant cultivars, particularly for Fusarium wilt (Tropical Race 4) and Black Sigatoka, two of the most destructive banana diseases.

#### **Fusarium Wilt Resistance**

The International Institute of Tropical Agriculture (IITA) has led efforts to combat Fusarium wilt by using wild Musa species that show natural resistance to the disease. These wild species have been crossed with commercial varieties, and marker-assisted selection (MAS) has been used to identify and select for resistance genes. As a result, the IITA has developed Fusarium-resistant banana varieties, which are undergoing field trials in Uganda and other banana-growing regions. This approach allows the rapid development of resistant cultivars without compromising other desirable traits, such as fruit quality and yield (Ortiz & Swennen, 2010).

#### **Black Sigatoka Resistance**

The National Banana Research Program (NBRP) in Ecuador has successfully utilized marker-assisted breeding to develop banana varieties resistant to Black Sigatoka. These banana varieties demonstrate enhanced resistance to the fungal disease, enabling higher productivity with reduced fungicide applications (Perrier *et al.*, 2019). The use of molecular markers linked to disease resistance traits has accelerated the process of developing these resistant cultivars.

#### **Biofortification Efforts (e.g., Vitamin A-Enriched Bananas)**

In regions where bananas are a staple crop, enhancing their nutritional content through **biofortification** has become a focus in breeding programs. Biofortified bananas, such as Vitamin A-enriched varieties, are particularly valuable in regions with high levels of Vitamin A deficiency.

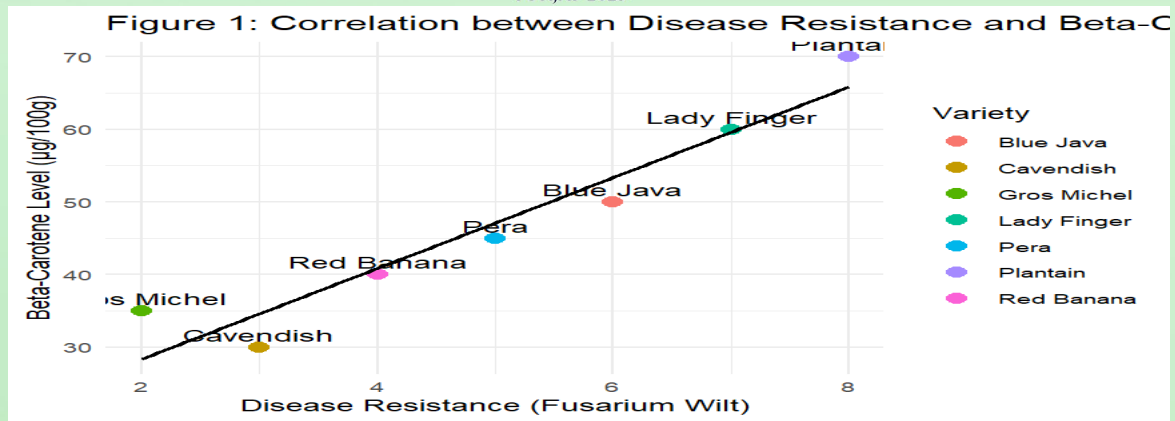
#### **Vitamin A-Enriched Bananas in Uganda**

In Uganda, a collaborative effort between the International Potato Center (CIP) and the National Agricultural Research Organization (NARO) has led to the development of Vitamin A-enriched bananas. These bananas have been biofortified to contain higher levels of pro-vitamin A carotenoids, primarily beta-carotene, which can be converted to Vitamin A in the body. The bananas developed show up to a 50% increase in beta-carotene content, providing an important tool for tackling Vitamin A deficiency in vulnerable populations. Field trials in Uganda have shown positive results, with farmers expressing interest in these varieties due to their health benefits (Okao *et al.*, 2019).

Figure 1 illustrates the relationship between disease resistance (specifically to Fusarium wilt) and beta-carotene levels in various banana varieties, highlighting the ongoing efforts in banana breeding programs. The x-axis represents disease resistance, with higher values corresponding to more resistant varieties, while the y-axis displays beta-carotene content (in micrograms per 100 grams of banana). The plot includes several commonly studied banana varieties, such as Cavendish, Pera, Red Banana, Gros Michel, Blue Java, Lady Finger, and Plantain.

The scatter plot shows the distribution of these varieties in terms of their resistance to Fusarium wilt and their beta-carotene levels. The regression line indicates the general trend, and the points represent individual varieties. While certain varieties show a strong correlation between improved disease resistance and higher beta-carotene content, others exhibit a more variable pattern. This data emphasizes the complexity in breeding for both disease resistance and biofortification, key goals in modern banana breeding programs aimed at improving both crop yield stability and nutritional quality."





Source: Okao *et al.*, 2029

### Future Perspectives and Research Gaps

Banana breeding is at the crossroads of several exciting innovations and challenges that could significantly transform the industry. To meet the growing demands for improved disease resistance, nutritional value, and yield, future research must focus on the integration of cutting-edge technologies, better understanding of the genetic makeup of banana, and improved access to these varieties by smallholder farmers. The following are key future perspectives and research gaps in banana breeding.

### Application of AI and Machine Learning in Banana Breeding

The integration of Artificial Intelligence (AI) and machine learning (ML) into banana breeding holds great promise for accelerating genetic improvement. These technologies allow for the analysis of large datasets, including phenotypic, genotypic, and environmental data, to predict the best-performing varieties based on specific traits. AI and ML algorithms can model complex relationships between different traits, enabling predictive breeding that reduces trial-and-error efforts in breeding programs. For instance, AI can help predict disease outbreaks, evaluate genetic diversity, and accelerate the identification of high-yield, disease-resistant varieties. While these technologies have shown great promise in other crops, their application in bananas is still in its early stages, highlighting the need for further development and adaptation to banana-specific breeding programs (Kumar *et al.*, 2022).

### Enhancing Genome Editing Precision

The advent of genome editing technologies like CRISPR/Cas9 has revolutionized the field of plant breeding, providing the ability to make precise genetic modifications. Genome editing tools have the potential to significantly enhance the disease resistance and nutritional quality of banana varieties, enabling faster development of biofortified and disease-resistant bananas. However, despite significant progress, the precision of genome editing tools needs further refinement to minimize off-target effects and improve the efficiency of genetic modifications. Researchers should also focus on expanding the toolkit to include base editing and prime editing, which offer greater precision in gene modifications (Zhao *et al.*, 2021). Additionally, regulatory challenges and public acceptance of genetically modified organisms (GMOs) in banana production need to be addressed to ensure the widespread application of genome editing technologies.

### Improving Smallholder Adoption of Improved Varieties

While significant progress has been made in developing improved banana varieties, the adoption of these varieties by smallholder farmers remains a critical challenge. Farmers, particularly in Sub-Saharan Africa and Southeast Asia, often face barriers to accessing improved banana cultivars, including high costs, lack of technical knowledge, and poor infrastructure for seed distribution. Moreover, many smallholder farmers are hesitant to adopt improved varieties due to concerns about market demand, seed availability, and the risk of crop failure. To overcome these barriers, future research should focus on improving seed distribution systems, enhancing farmer education programs, and conducting socio-economic analyses to ensure that improved varieties are economically viable for smallholder farmers. The adoption of community-based breeding programs and farmer-participatory approaches could help increase trust and participation in banana breeding programs (Mubiru *et al.*, 2020). Additionally, policies supporting subsidies for seed purchase and post-harvest technologies could help increase adoption rates.

### Closing Research Gaps

Despite advances in banana breeding, several research gaps remain. Understanding the genetic mechanisms underlying complex traits, such as disease resistance and drought tolerance, is still limited. More comprehensive genomic studies, particularly in under-researched banana species and cultivars, are required to identify genetic loci



associated with these traits. Furthermore, the banana microbiome plays a crucial role in the plant's health, and future research should explore how microbial communities affect disease resistance and nutrient uptake in bananas (Smith *et al.*, 2021). Finally, climate change is expected to alter banana production in many regions, making it imperative to study the potential impacts of climate stressors on banana growth and develop varieties resilient to changing environmental conditions.

## Conclusion

Banana breeding has made significant strides in recent years, thanks to advances in molecular tools and technologies. However, challenges such as long breeding cycles, limited genetic diversity, and the need for improved disease resistance continue to pose obstacles for the development of better banana varieties. This review has highlighted the importance of integrating molecular breeding techniques, such as marker-assisted selection (MAS), genome-wide association studies (GWAS), and genome editing tools like CRISPR/Cas9, to accelerate banana improvement programs. The application of these tools has not only enabled the development of disease-resistant varieties but has also opened new possibilities for biofortification, such as increasing the levels of beta-carotene and other essential nutrients in bananas.

## Acknowledgement

The authors thank the RSIF-PASET fund donors for supporting to publish this Article

## Conflict of interest

The authors declare no conflict of interest among them

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