

BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Achieving sustainable cultivation of bananas

Volume 2: Germplasm and genetic improvement

Edited by Professor Gert H. J. Kema

Wageningen University and Research, The Netherlands

Professor André Drenth, The University of Queensland, Australia



Contents

Series list	xi
Acknowledgements	xviii
Introduction	xix

1	An overview of genetic improvement in bananas over the last century	1
	<i>Mike Smith, Queensland Department of Agriculture and Fisheries, Australia; and Michael Pillay, Vaal University of Technology, South Africa</i>	
	1 Introduction	1
	2 Conventional breeding: evolutionary and reconstructive approaches	5
	3 Non-conventional breeding	9
	4 Marker-assisted selection and mapping	12
	5 Conclusion and future trends	15
	6 Where to look for further information	17
	7 Acknowledgements	18
	8 References	19

Part 1 Classification

2	Cytogenetics of structural rearrangements in Musa hybrids and cultivars	31
	<i>Fajarudin Ahmad, Indonesian Institute of Sciences (LIPI), Indonesia and Wageningen University & Research, The Netherlands; Peter M. Bourke and Henk Schouten, Wageningen University & Research, The Netherlands; Hugo A. Volkaert, Center for Agricultural Biotechnology - Kasetsart University, Thailand; Gert H. J. Kema, Wageningen University & Research, The Netherlands; and Hans de Jong, Kasetsart University, Thailand and Wageningen University & Research, The Netherlands</i>	
	1 Introduction	31
	2 Pollen fertility	33

3	Flow-cytometry	34
4	Fluorescent in situ hybridization (FISH)	34
5	Use of repetitive sequences in banana research	36
6	Chromosomal detection of single-copy sequences	38
7	Molecular markers in linkage studies	39
8	Case study	41
9	Conclusion and future perspectives	48
10	Where to look for further information	50
11	References	51
3	Identifying and classifying banana cultivars <i>Jeff Daniells, Queensland Department of Agriculture and Fisheries, Australia; and Steven B. Janssens, Botanic Garden Meise, Belgium</i>	59
1	Introduction	59
2	Banana cultivars: an overview	60
3	Classification at the genera level	61
4	Classification at the section level	65
5	Classification at the species level	66
6	Current banana cultivar groups	67
7	Identifying cultivars	72
8	Future trends and conclusions	77
9	Where to look for further information	78
10	References	79
4	Exploiting current <i>Musa</i> collections <i>V. Guignon, Alliance of Bioversity International and CIAT, France</i>	81
1	Introduction	81
2	Collections and accession conservation	84
3	Adding value to collection data	91
4	Data mining in collections	96
5	Case study	98
6	Summary and future trends	101
7	Where to look for further information	101
8	Acknowledgements	102
9	References	102
Part 2 Broadening the genetic base		
5	Scope of collecting wild <i>Musa</i> species germplasm <i>Julie Sardos, Alliance of Bioversity International and CIAT, Montpellier Office, France</i>	109
1	Introduction	109
2	Genetic vulnerability of banana plantations	110

3	The importance of crop wild relatives	111
4	Which wild <i>Musa</i> species to collect	113
5	How to collect wild <i>Musa</i> species	116
6	Theoretical and practical issues in collecting wild bananas	117
7	On the importance of capturing passport data and making them publicly available	123
8	Conclusion	125
9	Where to look for further information	125
10	References	127
6	Collection and evaluation of wild <i>Musa</i> species <i>Hugo A. Volkaert, Center for Agricultural Biotechnology - Kasetsart University, Thailand</i>	133
1	Introduction	133
2	The importance of collecting and characterizing wild <i>Musa</i> germplasm	134
3	An estimation of the genetic diversity missing from the <i>Musa</i> Germplasm Transit Centre (ITC)	136
4	Conserving cultivars versus wild germplasm	137
5	Conclusions	140
6	Where to look for further information	140
7	Acknowledgements	140
8	References	140
7	Collection and evaluation of banana and plantain landraces in Africa <i>D. Karamura and W. Ocimati, Bioversity International, Uganda; G. Blomme, Bioversity International, Ethiopia; J. G. Adheka, University of Kisangani (UNIKIS), Democratic Republic of the Congo; C. Sivirihauma, Université Catholique du Gabon (UCG), Democratic Republic of the Congo; D. B. Dheda, University of Kisangani (UNIKIS), Democratic Republic of the Congo; and E. Karamura, Bioversity International, Uganda</i>	143
1	Introduction	143
2	Overview of banana and plantain landraces in Africa	144
3	Banana and plantain landrace diversity and its conservation in Africa	147
4	Access to and utilization of characterization and evaluation data for banana and plantain landraces	153
5	Challenges for the maintenance and conservation of collected landraces in Africa	159
6	Recommendations for addressing challenges and exploiting opportunities for sustainable development of <i>Musa</i> landraces in Africa	162
7	Where to look for further information	162
8	References	164

8	Seed germination, preservation and population genetics of wild <i>Musa</i> germplasm	167
	<i>Bart Panis, Bioversity International and Katholieke University of Leuven (KUL), Belgium; Simon Kallow, Royal Botanical Gardens Kew, UK and Katholieke University of Leuven (KUL), Belgium; and Steven B. Janssens, Meise Botanic Garden, Belgium</i>	
	1 Introduction	167
	2 The importance of conserving crop wild relatives (CWRs)	168
	3 Seed morphology	170
	4 Seed germination	172
	5 Storage of wild <i>Musa</i> germplasm	177
	6 Population genetics of wild bananas	182
	7 Future trends and conclusion	184
	8 Acknowledgements	185
	9 Where to look for further information	185
	10 References	186
9	Safe dissemination of germplasm resources of banana	193
	<i>John Thomas, The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Ecosciences Precinct, Australia; Sébastien Massart, Integrated and Urban Plant Pathology Laboratory, Gembloux Agro-Bio Tech, University of Liège, Belgium; Ines Van den Houwe, Bioversity International Transit Centre, KU Leuven, Division of Crop Biotechnics – Laboratory of Tropical Crop Improvement, Belgium; Nicolas Roux, Bioversity International, France; and Kathy Crew, Queensland Department of Agriculture and Fisheries, Ecosciences Precinct, Australia</i>	
	1 Introduction	193
	2 General recommendations for international <i>Musa</i> germplasm exchange	194
	3 Germplasm from field collections	195
	4 Germplasm from <i>in vitro</i> collections	196
	5 Cryopreservation of <i>Musa</i> germplasm	198
	6 Seed banking	199
	7 DNA banking	199
	8 The need for virus indexing to ensure high health status	200
	9 Virus indexing of <i>Musa</i> germplasm	201
	10 Viruses integrated into the <i>Musa</i> genome	202
	11 Physical shipment of germplasm	202
	12 Regulations surrounding transfer	203
	13 Future trends in research	206
	14 Conclusion	208
	15 Where to look for further information	209
	16 References	210

Part 3 Genetic improvement through breeding

10	Making banana breeding more effective	217
	<i>F. Bakry, J. P. Horry and C. Jenny, CIRAD, UMR AGAP and AGAP, Université de Montpellier, CIRAD, INRAE, Institut Agro, France</i>	
	1 Introduction	217
	2 The need for sustainable genetic solutions	218
	3 Evolution and domestication resulting in gamete sterility of contemporary varieties and consequences for breeding	219
	4 Strategies in banana breeding	226
	5 Conclusion	248
	6 Where to look for further information	249
	7 Acknowledgements	250
	8 References	250
11	Overcoming the fertility crisis in bananas (<i>Musa</i> spp.)	257
	<i>Delphine Amah, International Institute of Tropical Agriculture (IITA), Nigeria; David W. Turner, The University of Western Australia, Australia; D. Jane Gibbs, Consultant, Australia; Allan Waniale, Makerere University and National Agricultural Research Laboratories, Uganda; Gil Gram, International Institute of Tropical Agriculture (IITA), Uganda and Katholieke University of Leuven (KUL), Belgium; and Rony Swennen, International Institute of Tropical Agriculture (IITA), Tanzania and Katholieke University of Leuven (KUL), Belgium</i>	
	1 Introduction	257
	2 Reproductive biology of banana	258
	3 Bract opening in banana	276
	4 Fertilization and seed set	283
	5 Embryo dormancy: a cause for poor germination?	288
	6 Parthenocarpy and sterility	289
	7 Conclusion and future trends	295
	8 Where to look for further information	297
	9 Glossary	297
	10 References	300
12	Targeted improvement of Cavendish clones	307
	<i>Eli Khayat, Rahan Meristem (1998) LTD., Israel</i>	
	1 Introduction	307
	2 Genetic background of bananas and plantains	308
	3 In vitro mutagenesis: somaclonal variation	309
	4 Conclusion and future trends	317
	5 References	318

13	Developing hybrid banana varieties with improved properties <i>Edson Perito Amorim, Vanusia Batista de Oliveira Amorim, Manassés dos Santos Silva, Fernando Haddad, Claudia Fortes Ferreira and Janay Almeida dos Santos Serejo, Embrapa, Brazil</i>	323
	1 Introduction	323
	2 Released hybrids	324
	3 Seeds as the basis of the genetic improvement program of Embrapa	326
	4 Improved diploids and their importance in the improvement of <i>Musa</i> spp.	326
	5 Phenotyping for resistance to Fusarium wilt race 1	328
	6 The Embrapa breeding cycle	330
	7 Alternatives to breeding based on crosses	333
	8 Future trends and conclusion	335
	9 References	336
14	Genetic modification of bananas: the long road to farmers' fields <i>James Dale, Queensland University of Technology, Australia; Wilberforce Tushemereirwe, National Agricultural Research Organisation, Uganda; and Robert Harding, Queensland University of Technology, Australia</i>	339
	1 Introduction	339
	2 Banana improvement by genetic modification and gene editing	341
	3 Case study: biofortified East African Highland bananas	354
	4 Future trends and conclusion	357
	5 References	359
15	The usage of phenotyping, genetics and functional genomics approaches to improve environmental stress factors in banana <i>Sebastien Christian Carpentier, Bioversity International and Katholieke University of Leuven (KUL), Belgium; and David Eyland, Katholieke University of Leuven (KUL), Belgium</i>	367
	1 Introduction	367
	2 Crop physiology and phenotyping	368
	3 Biotechnology to assist breeding	382
	4 Conclusions	386
	5 Where to look for further information	387
	6 Acknowledgements	387
	7 References	388
	Index	397

Introduction

Bananas are the world's most exported and valuable fruit. Banana production faces a number of challenges stemming from an extreme narrow genetic base on which commercial cultivation is based which leaves the crop highly vulnerable to a range of biotic and abiotic stresses. Due to the increasing global spread of banana diseases over the last 50 years there is an urgent need to widen the genetic base and incorporate disease resistance into new varieties while retaining or improving key yield and quality characteristics whilst also improving tolerances to abiotic stresses. This requires a thorough understanding of banana genetics that underlies any breeding effort, irrespective of the strategies that are being followed. These key issues are addressed in this volume

This book is the second in a three-volume series on bananas. Volume 1 published in 2018 focuses on the cultivation of bananas, this second volume is focused on germplasm and genetic improvement, and Volume 3 is devoted to pests and diseases. Each volume stands on its own but together we believe the three books provide a comprehensive review of all key aspects for a process towards sustainable banana production.

Volume 2 begins with an overview of the challenges faced trying in contemporary banana breeding programs. This is followed by an overview of the progress made in identifying and classifying *Musa* germplasm to broaden the genetic base as a foundation for a more sustainable banana industry in the future. Chapters review ways of classifying banana cultivars and exploiting current germplasm collections. Insights are provided into the wide genetic diversity in wild seeded *Musa* species and its poor representation in gene banks. This is followed by chapters on genetic improvement through breeding comprising a range of conventional breeding techniques, improving fertility, trait identification, new ways for developing hybrid varieties as well as several techniques aimed at achieving genetic improvement without hybridization.

Chapter 1 sets the scene by providing an overview of genetic improvement in bananas over the last century. Bananas were among the first crops cultivated by man and continue to be highly important for the livelihoods and food security of millions of people throughout the tropics and subtropics. Yet almost all banana varieties and landraces grown have been around for a very long time and some were selected in antiquity and clonally propagated ever since. The high level of sterility and parthenocarpy in bananas, producing edible fruit without seeds, has until the middle of the last century excluded genetic recombination as a means to select novel hybrids and achieve genetic gain. The fact that Cavendish and closely related varieties represent nearly half of the world's banana production has given rise to the extremely narrow genetic basis

for contemporary banana production as demonstrated by the emergence and subsequent spread of a range of pathogens, which underscores the genetic vulnerability of the global banana industry.

Resistance to diseases and pests is the best form of crop protection and it is imperative to develop new cultivars that perform well in changing growing environments. Currently, banana improvement – with relevant gains in resistance to diseases and pests – is accomplished through either conventional or non-conventional breeding strategies but compared to many other crops banana breeding is in its infancy. Various chapters critically review a variety of genetic improvement strategies that have been employed over the last century such as hybridization approaches, mutation breeding, somaclonal selection, genetic transformation and marker assisted selection. The role of genomics and more recent molecular breeding approaches are also discussed.

Part 1 Classification

The first part of the book begins with fundamental genetics in *Musa* and cultivars. Chapter 2 presents an overview of cytogenetics, genetics and genomics research carried out to elucidate the meiotic chromosome behaviour in the hybrids and the mapping of genes. Special attention is paid to the different origins of chromosomes and DNA sequences in the banana genomes. Finally, the chapter illustrates cytogenetic and linkage mapping of a diploid *M. acuminata* ssp. *malaccensis* using the diploid *M. acuminata* ‘Pahang’ as a reference genome and highlights the occurrence of structural rearrangements in the genome.

The next chapter focuses on identifying and classifying banana cultivars. Chapter 3 begins with an overview of different banana cultivars, followed by a discussion of the different classification methods and levels such as genera, sections and species. The chapter also highlights the current banana cultivar groups and examines how different cultivars can be identified and concludes with a summary of future research trends in the area.

In Chapter 4 current *Musa* germplasm collections are explored. They are a source of germplasm diversity which is the foundation for genetic improvement. However, the germplasm information systems aimed at increasing their utilisation and impact faced several challenges. The chapter discusses conservation and germplasm integrity, characterization of accessions and information systems that add value to collection documentation. Furthermore, it reviews information systems and their key role in collection management, with a focus on the importance of specific user interfaces and tools in order to simplify and accelerate research and breeding efforts relying on *Musa* germplasm. It considers a range of environmental and experimental data that play an important role in selection of accessions for breeding and

other purposes and a strategy is outlined for reducing gaps in collections using *in situ* banana collections.

Part 2 Broadening the genetic base

Part 2 opens with a chapter on the scope of collecting wild *Musa* species germplasm. The value of crop wild relatives as sources of diversity to secure crop production is increasingly recognized. In banana, past collecting and conservation efforts focused mainly on the two primary ancestors of cultivated bananas, *M. acuminata* Colla and *M. balbisiana* Colla. However, more than 70 taxa of wild *Musa* species exist in the wild and contain a vast array of genes which can be deployed in genetic improvement programs. Chapter 5 discusses the hitherto limited exploration of genetic diversity in wild bananas and how the industry focus on clonal propagation of the banana crop has discouraged the efficient collecting and conservation of wild *Musa* species. The change in focus from collecting only single clonal individuals to seed populations is presented and practical issues linked to collecting populations are discussed within the scope of wild *Musa* species reproductive biology.

Chapter 6 examines the collection and evaluation of wild *Musa* species consisting of about 70 different taxa distributed across the Asian tropics and subtropics from India, Southern China and Southern Japan in the north to Northern Australia in the south and the islands in the Pacific Ocean to the west. The chapter begins by highlighting the importance of collecting and characterizing wild *Musa* germplasm and provides an estimation of the genetic diversity missing in the *Musa* Germplasm Transit Center (ITC). It also discusses the importance of conserving cultivars versus wild germplasm, before concluding with a summary on why maintaining and expanding the ITC collection is so important in terms of conserving banana germplasm from all over the world.

Chapter 7 expands on topics touched upon in Chapter 6 and focuses on the collection and evaluation of banana and plantain landraces in Africa which is sometimes considered as a secondary centre of diversity for bananas. The chapter provides an overview of the different banana and plantain landraces present in Africa followed by an examination of landrace diversity and its conservation. It continues with discussing access to and utilization of characterization and evaluation data of banana and plantain landraces before highlighting the challenges of maintaining collected landraces in Africa. The chapter concludes with recommendations for addressing challenges and exploiting opportunities for the sustainable deployment of *Musa* landraces in Africa.

Chapter 8 covers seed germination, preservation and population genetics of wild *Musa* germplasm. Crop wild relatives play an important role

in modern plant breeding as they contain important traits for agriculture. Their conservation is therefore essential for future sustainable agriculture and food security. The chapter focuses on the different aspect and techniques that are required to establish seed collections of wild bananas and covers details of the physiology and morphology of banana seeds as well as difficulties encountered during germination of banana seed and putative solutions and possibilities for medium- and long-term maintenance. The chapter concludes with a summary of future research trends.

Chapter 9 reviews the safe dissemination of banana germplasm resources. It begins by examining the general recommendations for international *Musa* germplasm exchange, followed by methods to assure high health status germplasm from field and *in vitro* collections. The chapter also discusses cryopreservation of *Musa* germplasm, before reviewing seed and DNA banking. Due to sterility of bananas and the need to vegetatively propagate planting material, the need for virus indexing to ensure plant health is highlighted. The importance of indexing *Musa* germplasm for existing and novel viruses is discussed, particularly related to Banana Streak Virus, which can be integrated into the banana B genome. The chapter also covers practical aspects related to physical shipment of germplasm and the legal regulations surrounding international germplasm transfer and concludes with highlighting future research trends.

Part 3 Genetic improvement through breeding

The final part of the book starts with a discussion on approaches which can be used to improve banana breeding. A case is made for more genetically diverse bananas which meet specific demands such as market acceptable fresh fruit, cooking bananas or varieties dedicated to processing. Chapter 10 highlights the need for sustainable genetic solutions, followed by an evolutionary analysis of *Musa* species and the events that gave rise to gamete sterility producing the contemporary edible banana fruit. The chapter also discusses different breeding strategies to achieve genetic gain and gives an excellent overview of the difference between 'pedigree breeding' and 'reconstructive breeding' strategies and their perspective towards achieving future breeding goals.

Chapter 11 deals with overcoming the fertility crisis in bananas. Edible bananas are normally parthenocarpic and seedless, a condition which ensures edible fruits, but this infertility limits the use of existing varieties in crosses to generate new combinations of targeted traits for crop improvement. Hybridizations involving edible bananas result in very few hybrid seeds with low or no viability. The inability to effectively use existing varieties with desired traits as parents for new crosses and backcrosses constitutes a fertility crisis which seriously hampers progress in banana breeding. The chapter discusses

key processes surrounding effective pollination, fertilization and viable seed production in relation to our hitherto knowledge concerning banana reproductive biology. For instance, it explores possible limitations of seed-set and provides insights to overcome the fertility crisis towards a much-needed renaissance in banana breeding, which would enable gene recombinations through intra- and interspecific crosses.

Chapter 12 focuses on targeted improvement of Cavendish clones. Despite hundreds of different available banana cultivars, growers and the banana export industry prefer cultivars from the Cavendish subgroup. All Cavendish cultivars are triploid and parthenocarpic. Therefore, improvements depend on somaclonal variation which has resulted in a diverse range of morphological phenotypes. Through clonal propagation and more recently the development of *in vitro* culture, preferred clones can be rapidly mass propagated and conveniently offered to growers. The chapter outlines the potential of ongoing selection and genetic improvement technologies that are presently available and how these have been utilized to improve the performance of Cavendish cultivars to overcome today's biological and environmental challenges.

Chapter 13 concentrates on developing hybrid varieties with improved properties, specifically focusing on research undertaken at Embrapa in Brazil. It opens with a discussion concerning the background of the 11 hybrid banana cultivars that have been released by Embrapa, followed by a review of using fertile seeded germplasm as a basis of the breeding strategy. The chapter outlines the development and use of improved diploids - containing multiple desirable traits - in the process of breeding sterile banana cultivars. Resistance breeding to *Fusarium* wilt race 1 is discussed, followed by a review of Embrapa's breeding cycle and some alternative methods to classical crossbreeding strategies. The chapter concludes with the importance of additional research to support the banana breeding effort.

The great challenge for bananas into the future is to develop cultivars with high agronomic acceptability and yield potential, possessing multiple disease resistances and abiotic stress tolerances, a high level of transportability and enhanced fruit quality. In short, the two major genetic improvement strategies are conventional breeding, delivering new cultivars, and genetic modification, delivering improved current cultivars. Chapter 14 reviews banana improvement through the process of genetic modification and gene editing with a focus on the journey required for these improved varieties to reach farmers' fields. The chapter contains a case study on biofortified East African Highland bananas and concludes with an overview of future research trends.

Chapter 15 addresses the development and use of rapid phenotyping methods, genetics and genomic selection approaches for tolerance to environmental stress factors in banana. This final chapter discusses banana

physiology in relation to its agro-ecological environment and the phenotyping of traits such as tolerance to sub-optimal temperature water availability. A workflow is presented to phenotype for these traits in a high throughput-controlled environment. Once interesting traits are identified, strategies are outlined to identify potential molecular markers that are closely linked to genes encoding for these traits, including genome-wide association studies (GWAS), associative transcriptomics and proteomics. The chapter concludes with highlighting how correlations between phenotype and genetic markers will aid future banana breeding.

Taken together, the chapters in this volume show the challenges of genetic improvement of bananas due to the inherent fertility issues and the lack of basic genetic research which underpins breeding. At the same time, all chapters also highlight the urgent need for genetic improvement, specifically for disease resistance, and in general call for broadening the genetic basis of the current banana industry. To achieve this available *Musa* germplasm must be collected, protected *in situ*, moved around the world in a safe manner, safeguarded, and characterised for the presence of useful traits. The underlying genetics needs to be unravelled and enabling technologies for either direct or indirect select for these traits need to be developed. Novel approaches in genetic improvement and breeding as put forward and scrutinized in this volume await deployment to bypass the lack of fertility in edible banana varieties. A truly sustainable future for bananas lies in a coordinated, synergistic and multidisciplinary combination of approaches from a production, an employment, a logistic and consumer perspective. Future banana production entails a smooth integration of germplasm selection, breeding, agronomy and pre- and post-harvest integrated pest and disease management.

Chapter 1

An overview of genetic improvement in bananas over the last century

*Mike Smith, Queensland Department of Agriculture and Fisheries, Australia; and
Michael Pillay, Vaal University of Technology, South Africa*

- 1 Introduction
- 2 Conventional breeding: evolutionary and reconstructive approaches
- 3 Non-conventional breeding
- 4 Marker-assisted selection and mapping
- 5 Conclusion and future trends
- 6 Where to look for further information
- 7 Acknowledgements
- 8 References

1 Introduction

Bananas were among the first crops cultivated by man and continue to be important for the livelihoods of millions of people throughout the tropics and subtropics. Yet many cultivars and landraces are susceptible to biotic and abiotic stresses. Genetic resistance to diseases and pests is the best form of crop protection and it is imperative to develop new cultivars that perform well across a variety of changing environments. Currently, the production of improved cultivars with relevant gains in resistance to diseases and pests and other preferred agronomic traits has been accomplished through both conventional and non-conventional breeding. This chapter reviews the various genetic improvement strategies used to develop new banana cultivars and discusses the strategies that have been employed over the last one hundred years. They include hybridization approaches, mutation breeding, genetic transformation and marker-assisted selection. The role of genomics and more recent molecular breeding approaches are also discussed.

Vegetatively propagated crops, such as banana, play an important role as a source of food and income especially for small-scale farmers in many countries. Banana is also a vital export crop for a number of countries and to which it contributes a large part of the gross domestic product (GDP). Like many other

crops, bananas are also affected by a number of diseases and pests (Table 1) that are responsible for severe yield losses in both small-scale and large commercial plantations. Therefore, genetic improvement of the crop is essential to mitigate current and envisaged biotic and abiotic stresses. However, almost exclusively, farmers were and are still cultivating a small number of clones selected by their forebearers, some of which extend back thousands of years.

Banana breeding began in 1922 in Trinidad and four years later in Jamaica with the singular objective of developing a *Fusarium* wilt-resistant 'Gros Michel' (AAA) export dessert banana (Simmonds, 1966; Shepherd, 1974). Initially, banana breeding was considered a paradox since most of the commercially important cultivars are parthenocarpic triploids ($2n = 3x = 33$) and largely sterile. Seedlessness of the fruit is an essential characteristic that consumers

Table 1 Resistance to major banana diseases and pests are objectives of most conventional and non-conventional breeding programmes

Pathogen or pest	Crop effect	Genetic resistance identified
<i>Fusarium</i> wilt (various <i>Fusarium</i> spp.) race 1	Wilting and death of susceptible cultivars	Yes*
<i>Fusarium</i> wilt (<i>Fusarium odoratissimum</i>) TR 4	Wilting and death of susceptible cultivars	Yes*
Black Sigatoka (<i>Pseudocercospora fijiensis</i>)	Loss of green leaf; severely reduced yields; very poor shelf life of fruit	Yes
Yellow Sigatoka (<i>Pseudocercospora musae</i>)	As above, but usually not as severe	Yes
Xanthomonas wilt (<i>Xanthomonas campestris</i> pathovar <i>musacearum</i>)	Internal discolouration and rotting of fruit leading to a 'top-down' infection of plant	Incomplete resistance
Moko; Bugtok (<i>Ralstonia solanacearum</i> biovar 1)	Internal discolouration and rotting of fruit leading to a 'top-down' infection of plant	Incomplete resistance
Blood disease (<i>Ralstonia syzygii</i> subsp. <i>celebesensis</i>)	Internal discolouration and rotting of fruit leading to a 'top-down' infection of plant	Incomplete resistance
Banana Bunchy Top Virus (BBTV)	Severe stunting of plants leading to crop failure	None
Nematodes (<i>Radopholus similis</i> , <i>Pratylenchus goodeyi</i> , <i>Helicotylenchus multicinctus</i>)	Damage to roots leading to toppling and reduced yield	Yes*
Banana weevil (<i>Cosmopolites sordidus</i> , <i>Castniomera licus</i>)	Boring and damage to rhizome and stems leading to snapping and reduced yield	None

* Complete resistance has been identified as well as levels of partial resistance.

demand and is a trait that must be retained by the breeder. In order to generate new cultivars, breeders need to conduct crosses that produce seeds from which to grow plants and make their selections. Despite the purported sterility of most cultivars, banana breeding turned out to be possible and was initially based on the ability of 'Gros Michel' and its shorter natural mutants to produce seeds at a very low frequency, for example, there was an average of two seeds per bunch when hand pollinated with wild diploids ($2n = 2x = 22$) (Simmonds, 1966). It was discovered that 'Gros Michel' contributed an unreduced triploid gamete that yielded tetraploids ($2n = 4x = 44$) when fertilized with pollen from a diploid.

Although Fusarium wilt-resistant tetraploid hybrids were produced by the Imperial College of Tropical Agriculture (Trinidad), other undesirable attributes such as poor shelf life, short fingers or weak pedicels meant that none of the hybrids was commercially acceptable (Stover and Simmonds, 1987). Cavendish, selected in antiquity by farmers in the centre of origin of banana in Southeast Asia, was reported to be resistant to *Fusarium* spp. (so-called race 1 strains), which caused the Panama disease or Fusarium wilt epidemics in 'Gros Michel' and eventually replaced it in the international trade.

Today there are twelve banana-breeding programmes around the world that differ in their objectives and breeding strategies, while addressing distinct regional needs. Most of these programmes have been reviewed recently (Tenkouano et al., 2011b; Ortiz and Swennen, 2014) and their key characteristics are outlined in the next section. While resistance to Fusarium wilt (especially to the devastating tropical race 4 - TR4) remains a priority, resistance to Sigatoka leaf diseases (*Pseudocercospora fijiensis* and *P. musae*) and a complex of plant-parasitic nematodes (*Radopholus similis*, *Pratylenchus goodeyi* and *Heliocotylenchus multicinctus*) are also important breeding objectives (Table 1). With this in mind, breeders aim to develop resistant cultivars that retain acceptable organoleptic properties and fruit quality. Recently, fruit quality traits such as increased pro-vitamin A has gained prominence as an objective for banana breeding (Amah et al., 2019a,b,c). Hybrids should also be productive over a wide range of conditions and have a short stature that is easy to manage and harvest.

Recent breakthroughs in banana breeding have overturned earlier attitudes to breeding strategies. Aguilar Morán (2013) showed that it was possible to improve the triploid Cavendish cultivar through conventional breeding as they are not as sterile as previously considered. The rate of seed production was approximately one seed for every 100 pollinated bunches. Advances in genetic marker and sequencing technologies have allowed the development of tools to decipher the complexity of the *Musa* genome and significantly increase the insights into the genetic make-up of our banana cultivars, which will vastly improve the efficiency of banana breeding. Bakry and Horry (2016) have shown

that only a few hybridization events have occurred to produce our present-day cultivars from their wild, seedy ancestors. Molecular markers can now be utilized to best select parents that combine fertility with other desirable traits to mimic the sequence of crosses that occurred naturally over several millennia (Bakry and Horry, 2016). This approach is particularly important in a biological context of gamete sterility in desirable but seedless cultivars and difficulties to get large quantities of seeds for which recombination and introgression of desirable genes remains the greatest challenge.



Figure 1 Conventional breeding sometimes involves hand pollinating hundreds of bunches (above) to recover a few seeds (below), which are usually embryo-cultured to obtain plants for further rounds of selection and breeding. (Picture courtesy of International Institute of Tropical Agriculture, Uganda).

Currently, the production of improved cultivars with relevant gains in resistance to diseases and pests and other preferred agronomic traits has been accomplished through both conventional and non-conventional breeding. In conventional breeding hundreds of bunches have often been hand-pollinated to obtain a few seeds (Fig. 1); however, some crosses have been more productive and have accelerated genetic improvement goals. These various strategies are discussed in the next section.

2 Conventional breeding: evolutionary and reconstructive approaches

Tenkouano et al. (2011b) discuss two competing breeding philosophies, which however, are not mutually exclusive. Evolutionary breeding, which many breeding programmes referred to as the conventional approach, attempts to mimic the evolutionary development of the *Musa* species complex. In this process, female fertile triploids (AAA, AAB or ABB genomes) with good agronomic and fruit characteristics are crossed with diploid accessions of *M. acuminata* (AA) or *M. balbisiana* (BB), which often are the sources of resistance genes the breeders seek. The $3x/2x$ crosses produce primary hybrids with large phenotypic variation subtended by ploidy and genome polymorphisms, among which diploids and tetraploid progenies that are disease resistant and agronomically desirable are selected and intermated by way of $4x/2x$ crosses to produce secondary triploid hybrids.

The second school of thought may be described as reconstructive breeding, whereby breeders attempt to recreate the landraces by first determining their most likely ancestors from the pool of diploids available and subsequently using only such putative or closely related derivatives in crosses aiming at synthesizing improved variants of the susceptible landraces. This approach advocated by Stover and Buddenhagen (1986) involves the use of colchicine to double the chromosome number of natural and improved diploids in order to induce the formation of tetraploids, which are crossed with other diploids leading to the synthesis of sterile triploid individuals.

The earliest banana-breeding programme, set up at the Imperial College of Tropical Agriculture (Trinidad), is no longer operational. The honour of being the oldest continuous banana-breeding programme in operation is that of Fundación Hondureña de Investigación Agrícola (FHIA), which was established in 1959 by the United Fruit Company in La Lima, Honduras, to develop a Fusarium wilt-resistant 'Gros Michel' (Rowe, 1987; Rowe and Rosales, 1994). FHIA's programme favoured an evolutionary approach that initially focussed on developing tetraploid dessert bananas, but gradually expanded to produce plantain and cooking bananas. Rowe and Rosales (1994) stated that the breeding objectives of FHIA by the latter part of the twentieth century were

Index

- 1T4/4T1 translocation 47–48
- 2,3,5-triphenyl tetrazolium chloride 271
- 2n gametes production 234

- AAAA neo-autotetraploids 239
- AAA hybrid bananas Breeding 243–245
- AAA Mutika 232
- AABB neo-allotetraploids 238
- ABB-cooking bananas 270
- Agrobacterium*-mediated protocol 11
- Akondro Mainty 278
- Androecial (stamens) 259
- Anthesis 259
 - pollen viability 271–272
 - stigma receptivity 270–271
- API. *see* Application programming interface (API)
- Application programming interface (API) 96
- Arabidopsis* 274, 295
- Asynapsis 264
- Australimusa 65–66
- Auxins 289

- BAC. *see* Bacterial artificial chromosome (BAC)
- Bacterial artificial chromosome (BAC) 38–39
- Bakry personal observations 225
- Banana and plantain landraces, Africa
 - diversity and conservation 147–153
 - maintenance and conservation challenges 159
 - genetic diversity reduction 160
 - limited investment, effective management for 160–161
 - political and social upheavals 161
 - poor documentation and poor-quality data 159–160
 - supportive policies, lack of 161
- national *Musa* collections 149–152
- overview 144–147
- sustainable development,
 - recommendations for 162
- utilization data access 153–154
 - characterization data 156–157
 - data access and availability 158–159
 - evaluation data 157–158
 - passport data 154–156
- Banana B genome 202
- Banana black leaf streak fungus (*Pseudocercospora fijiensis*) 218
- Banana breeding 217–250
 - strategies in 226
- Banana crossing strategies 238
- Banana cultivars
 - classification
 - genera level 61–65
 - section level 65–66
 - species level 66–67
 - groups 67–71
 - identifying, process of 72–77
 - overview 60–61
- Banana Genome Hub (BGH) 83
- Bananas
 - evolution of 222–223
 - Fusarium wilt of 194
 - germplasm resources of 193–210
 - improve cooking 246–248
 - overcoming fertility crisis 257–297
 - reproductive biology 258–276
 - safe dissemination of 193–210
 - seed testing 207–208
 - shoot tips 198
 - streak disease 200
 - in vitro embryo rescue in 225
- Banana streak viruses (BSVs) 200
- BanMMV 201
- Barley yellow dwarf virus 207

- BBrMV 201, 207
 BBTV 201
 BGH. *see* Banana Genome Hub (BGH)
 Biofortification 354-356
 Biotechnology breeding 382
 genetic modification and genome editing 385-386
 genomics, SNP technology and genome-wide association studies 383-385
 outcome 386-387
 Bioversity-CIAT Alliance 209
 Bivalents 264
 Black Sigatoka 6-7
 Bluggoe 264, 286
 Bobby Tannap 270
 Bract lift 282
 Bract opening
 in banana 276
 diurnal behaviour of 276-278
 glasshouse and field 278-279
 and insect visitation 282-283
 pollination in *Musa* species 283
 process of 276
 sunrise and sunset 279-281
 Bracts curl 279, 282
 Brazilian Agricultural Research Corporation (Embrapa) 323-324
 characteristics of 325
 female and male parents 327
 new generation of 330
 see also Hybrid varieties
 'Breeding by design' approach 245
 Breeding programme 5-9
 BSMYV 208
 BSVs. *see* banana streak viruses (BSVs)
- Calcutta 4 112, 228, 236, 273, 286
 Callimusa 66
 CARBAP 232. *see* Centre Africain de Recherche sur Bananiers et Plantains (CARBAP)
 CARBAP 832 232
 CARBAP K74 232-233
 Carpel 266
 CAT2. *see* Coding for the catalase enzyme (CAT2)
 Cavendish 217
 Cavendish-like banana hybrids 245
 Centre Africain de Recherches sur les Bananiers et Agriculture (CARBAP) 7-8
 Centre Africain de Recherche sur Bananiers et Plantains (CARBAP) 230
 Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) 7-8
 CGIAR. *see* Consultative Group for International Agricultural Research (CGIAR)
 Cinnus 259
 CIRAD 916 244
 CIRAD 918 244
 CIRAD 920 244
 CIRAD 925 244
 CMV 201, 208
 Coding for the catalase enzyme (CAT2) 136-137
 Conserving crop wild relatives (CWRs) 168-170
 Consultative Group for International Agricultural Research (CGIAR) 205
 Conventional breeding 5-9
 CRBP39 231
 CRISPR/Cas9 technology 12-13
 Crop physiology and phenotyping
 floral phase 368-369
 fruit phase 369
 light 377-382
 temperature 375-377
 vegetative phase 368-369
 water 370-375
 Crop Wild Relatives (CWR) 111
 Cross-breeding 226
 Cryopreservation 199
 Cultivar process identifying 72-77
 CWR. *see* Crop Wild Relatives (CWR)
 Cytogenetics of structural rearrangements case study 41-43
 genotyping and comparison, genetic and physical maps 44-45
 linkage analysis 45-48
 chromosomal detection, single-copy sequences of 38-39
 molecular markers, linkage studies in 39-41
 repetitive sequences, use of 36-38
- DArTs. *see* Molecular markers
 DArTseq platform 44
 Detection protocols 202
 Diseases resistance 1-2
 Distribution of plantain 154
 DNA banking 199-200
 Domestication 194
 Dominant genes (*Pi*) 292
 Doubled AA diploids 237

- Double-diploid parthenocarpic clones 237
 'Droplet vitrification' method 199
- EAHB. *see* East African Highland Banana (EAHB)
- East African Highland Bananas (EAHB) 230, 270-271
- eBSV. *see* Endogenous banana streak virus (eBSV)
- eBSV-containing B genome cultivars 208
- Edible varieties 221-223
- Embryo dormancy 288-289
- Embryos cryopreservation 179
- Endogenous banana streak virus (eBSV) 8-9, 231
- Ensete* 267
- Eumusa 65
- Europe problem 340-341
- Exploiting *Musa* collections
 accession conservation
 characterization 89-90
 conservation strategies 88-89
 field verification 90-91
 overview 84-88
 adding value
 environmental data 93
 experimental data 91-93
 integrating in situ data 95-96
 measuring diversity 93-95
 case study 98-101
 data mining
 building data bridges 97-98
 dedicated user interfaces 97
 information systems 96
 future trends 101
 research ordering 98
- F2 hybrid progenies 231
- FDR. *see* first-division restitution (FDR)
- Fertilization and seed set 283-287
- FHIA. *see* Fundación Hondureña de Investigación Agrícola (FHIA)
- FHIA-21 231
- Field capacity 370
- Field collections 195
 disadvantage 196
 factors 196
- Field gene banks 160-161
- Field verification process 90-91
- 'Figue' cluster 243
- First-division restitution (FDR) 227
- FISH. *see* Fluorescent in situ hybridization (FISH)
- Flow-cytometry 34
- Fluorescent in situ hybridization (FISH) 34-36
- Fundación Hondureña de Investigación Agrícola (FHIA) 5-6, 228
- Fusarium oxysporum* spp. *cubense* TR4 218
- Fusarium* spp. 3
- Gamete sterility
 consequences for breeding 219-225
 of contemporary varieties 219-225
 and low seed-set handicap
 breeding 223-225
- Gametogenesis 262
 androecium, The 268-269
 gynoecium 266-267
 issue in banana 262-266
 pollen production 269
 style, the 267
- Gametophytic self-incompatibility 285
- GDD. *see* Growing degree days (GDD)
- Genetic analysis 45
- Genetic improvement
 breeding 217-249
 overview 1-5
- Genetic modification 341, 345, 357-358
- Genome editing techniques 12, 346-347
- Genome-wide association analysis 295
- Genome-wide association studies (GWAS) 14, 383-384
- Genomic in situ hybridization (GISH) 35
- Germplasm
 from field collections 195-196
 from *in vitro* collections 196-198
- Germplasm, physical shipment of 202-203
- Germplasm Health Unit (GHU) 207
- Germplasm transfer 205
- GHU. *see* Germplasm Health Unit (GHU)
- Gibberellins 289
- GISH. *see* Genomic in situ hybridization (GISH)
- Grand Naine 273
- Growing degree days (GDD) 376
- GWAS. *see* Genome-wide association studies (GWAS)
- Gynoecial (pistils) 259
- Hand pollination 283
- Heterozygosity 223
- High Throughput Sequencing (HTS) 206
- Histidine kinase CK11 295
- Hormonal balance 292
- HTS. *see* High Throughput Sequencing (HTS)
- Huti White 278

- Hybrid varieties
 breeding cycle 330-333
 crosses
 chromosomes duplication 333-334
 future trends 335-336
 somaclonal variation 334-335
 cultivars 324-326
 genetic improvement program 326
Musa spp. 326-328
 overview 323-324
 resistance phenotyping 328-330
- Hydraulic conductivity 372
- ICNCP. *see* The International Code of Nomenclature for Cultivated Plants (ICNCP)
- IITA. *see* International Institute of Tropical Agriculture (IITA)
- Immunocapture-PCR 242
see also Molecular markers
- IMTP. *see* International *Musa* Testing Programme (IMTP)
- Information system 82-84
- Institut pour la recherche en développement (IRD) 199
- International Institute of Tropical Agriculture (IITA) 230
- International *Musa* germplasm exchange
 general recommendations for 194
 IPPC 195
 ISPM 195
- International *Musa* Germplasm Transit Center (ITC) 83-84, 98-101
- International *Musa* Testing Programme (IMTP) 83, 139
- International Plant Protection Convention (IPPC) 195, 202
- International Standards for Phytosanitary Measures (ISPM) 195
- International Treaty on Plant Genetic Resources for Food and Agriculture 205
- International Union for Conservation of Nature (IUCN) 115
- Interspecific diploids 224
- In vitro Musa* accessions 197
- In vivo* pollen growth 286
- IPPC. *see* International Plant Protection Convention (IPPC)
- ISPM. *see* International Standards for Phytosanitary Measures (ISPM)
- ITC. *see* International *Musa* Germplasm Transit Center (ITC)
- IUCN. *see* International Union for Conservation of Nature (IUCN)
- Kawanda Research Station 161
- 'Khai' cluster 243, 245
- Kisukari Mchare 278
- Kunnan (Indian AB landrace) 239
- 'Kunnan' landrace (ABcv) 241
- 'Kunnan' varieties (ABcv) 226
- Lacatan 245
- LAMP. *see* Loop Mediated Isothermal Amplification (LAMP)
- Leaf growth 374-375
- Loop Mediated Isothermal Amplification (LAMP) 201
- Male meiotic restitution 265
- Marau 269
- Marker-assisted selection 242
- Marker selection and mapping 12-15
- Matching pollination 276
- Mature embryo sacs 266
- Mbi Egome 269
- Mchare 222
- 'Mchare' AACv accessions 245
- Mchare Mlelembo 278
- Mealybug-transmitted viruses 200
- Meiosis avoidance 265
- Mendelian principles 288
- Meristems 198
see also banana shoot tips
- MGIS. *see* *Musa* Germplasm Information System (MGIS)
- MGIS Online Ordering System (MOOS) 83, 98, 101
- MGIS tools 93-96
- Molecular markers 197
- MOOS. *see* MGIS Online Ordering System (MOOS)
- Morphological dormancy 176
- Musa acuminata* spp. 15, 66-67, 199, 219
- Musa balbisiana* 68-69, 199, 219
- MusaBase 99
- Musaceae 62-64
- Musa* datasets 16-17
- Musa* genome
 safe movement of 203
 viruses integrated 202
- Musa* germplasm, Cryopreservation of 198-199
- Musa* Germplasm Information System (MGIS) 72, 82-83, 97-101, 209

- Musa* Germplasm Transit Centre (ITC) 136-137
- MusaID 76
- MusaNet* Global Survey of *Ex Situ* Collections 196
- Musa* (AAB) plantain 'Bobby Tannap' 261-262
- Nagoya Protocol 205
- NARO 232
- NARO. *see* National Agricultural Research Organisation (NARO)
- National Agricultural Research Organisation (NARO) 230
- National Plant Protection Organisation (NPPO) 203
- National Research Center for Banana (NRCB) 8, 228
- Nematodes 353
- Next-generation sequencing (NGS) 95, 101, 206
- n gamete donor 223
- NGS. *see* Next-generation sequencing (NGS)
- Non-chimeric AAAA autotetraploids 236
- Non-chimeric AABB allotetraploids 236
- Non-conventional breeding
genetic transformation 10-12
somaclonal variation and mutation breeding 9-10
- NPPO. *see* National Plant Protection Organisation (NPPO)
- NRCB. *see* National Research Centre for Banana (NRCB)
- Obino l'Ewai 270
- On-farm conservation 161
- Organogenesis 259
- Ovules per flower (Of) 287
- Paclobutrazol 292
- Parthenocarpic edible diploids 228
- Parthenocarpy and sterility 289-295
an adaptive feature 294-295
gene linked 295
phenotypic relationship 291
- (RT)-PCR tests 207
- Pedigree breeding 226-233
- Phytoplasma screening 207-208
- Pisang Awak (ABB) 224
- 'Pisang Klutuk Wulung' BB accession 242
- Pisang lilin 273, 293
- 'Pisang Mas'/'Sucrier' (AAcv) 226
- PITA 14 231
- 'Pointe d'Or' brand 244
- Pollen fertility 33-34
- Pollen guidance 273-274
- Pollen interaction 272-273
- Pollen-pistil interaction 271
- Pollen preservation 284
- Pollen stainability 272
- Pollen staining 43
- Pollen tube growth inhibition 285
- Pollination success 286
- Pollination techniques 283-285
- Pome group (AAB) 273
- Popoulu AAB clones 224
- Population genetic approaches 182
- Prata* clones 323-325
- Pre-breeding approach 247
- Primary tetraploids 226-231
- Problem with bananas 339-340
- Progression to field trials 349-354
- ProMusa 78
- ProMusa website 209
- Prunus* 207
- Pulp development 289
- Quantifying fertility in *Musa* 286-287
- Radiation 371
- Radopholus similis* 228
- Rasthali 273
- RCA. *see* Rolling Circle Amplification (RCA)
- Reconstructive breeding 5, 233-248
examples of 240-241
principle 234
process of 234-237
- Reconstructive Breeding 226
- 'Red' triploids 264
- Regulations 203-206
- Reproductive biology 260
- Rhodochlamys 65
- Rolling Circle Amplification (RCA) 201
- Root systems 374
- Scoring system 68-69
- SDR. *see* second-division restitution (SDR)
- Season-dependent seed set 281
- Secondary triploids 231-233
- Second-division restitution (SDR) 227
- Seed banking 199
- Seedless 291
- Seedlessness 292
- Seed potential 224

- Seeds Distribution 274
 quantifying 274-276
- Self-pollination 284
- Seminiferous banana 221
- Slow growth storage 198
- SMTA. *see* Standard Materials Transfer Agreements (SMTA)
- Somaclonal variation 10
- Southern-blot 242
 see also Molecular markers
- SSR. *see* Molecular markers
- Stage III stigmas 270
- Stage II stigmas 270
- Stage I stigmas 270
- Stage IV stigmas 270
- Standard Materials Transfer Agreements (SMTA) 205
- Stenospermocarpic 289
- Stomatal regulation 372-373
- Strelitzia reginae* 267
- Structural heterozygosity 224
- Sultana (*Vitis vinifera*) 289
- Sustainable genetic solutions 218-219
- Sweet-acid bananas 241-242
- Switch gene (*P1*) 292
- Tamil Nadu Agricultural University (TNAU) 228
- Targeted improvement, cavendish clones of
 future trends 317-318
 genetic background 308-309
 overview 307-308
 in vitro mutagenesis
 analysis 313
 components 309-310
 demethylation 315-116
 genetic modifications 316-317
 genetic pathways 311-312
 long terminal repeat (LTR)
 312-313
 methylation 313
 mutations 310-311
- Taxonomy 59-60
- Temperatures germination 174-175
- Tetraploidy 226
- The International Code of Nomenclature for Cultivated Plants (ICNCP) 60
- TNAU. *see* Tamil Nadu Agricultural University (TNAU)
- Traits, transgenes and genomes 347-349
- Transgenic bananas 342-345
- Triploidization events 223
- Triploids 221
- Tuu Gia 269
- Understanding seed set 285-286
- Vapour pressure deficit (VPD) 371
- Virus indexing
 to ensure high health status 200-201
 of *Musa* germplasm 201-202
- VPD. *see* Vapour pressure deficit
- Wild *Musa balbisiana* (BBw) 198
- Wild *Musa* species germplasm 219-221
 capturing passport data 123-125
 collection and evaluation
 vs. conserving cultivars 137-139
 genetic diversity, estimation of
 136-137
 importance of 134-136
 conserving crop wild relatives (CWRs)
 importance 168-170
 distribution, diversity and
 taxonomy 113-115
 future trends 184-185
 genetic vulnerability 110-111
 importance of crop 111-113
 material to collect 116-117
 overview 167-168
 population genetics of 182-184
 prioritizing 115-116
 seed germination 172-173
 alternating temperature
 requirements 174-175
 coat and structures, role of 175-176
 collection 173-174
 embryo development 176
 outcome 176-177
 seed morphology 170-172
 storage of 177-178
 embryos, conservation of 178-179
 outcome 181-182
 pollen, conservation of 181
 seeds, conservation of 179-181
 theoretical and practical issues
 implication 122-123
 pollinators 118-120
 reproductive biology 118
 seed dispersers 120-121
- Yangambi KM 5 286
- Zingiberales 267