BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Achieving sustainable cultivation of bananas

Volume 2: Germplasm and genetic improvement

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SCIENCE PUBLISHING

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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 a 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

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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

http://dx.doi.org/10.19103/AS.2020.0070.20

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
Fusarium wilt (various *Fusarium* spp.) race 1	Wilting and death of susceptible cultivars	Yes*
Fusarium wilt (*Fusarium odoratissimum)* TR 4	Wilting and death of susceptible cultivars	Yes*
Black Sigatoka (*Pseudocercospora fijiensis*)	Loss of green leaf; severely reduced yields; very poor shelf life of fruit	Yes
Yellow Sigatoka (*Pseudocercospora musae*)	As above, but usually not as severe	Yes
Xanthomonas wilt (*Xanthomonas campestris* pathovar *musacearum*)	Internal discolouration and rotting of fruit leading to a 'top-down' infection of plant	Incomplete resistance
Moko; Bugtok (*Ralstonia solanacearum* biovar 1)	Internal discolouration and rotting of fruit leading to a 'top-down' infection of plant	Incomplete resistance
Blood disease (*Ralstonia syzygii* subsp. *celebesensis*)	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 (*Radopholus similis, Pratylenchus goodeyi, Helicotylenchus multicinctus*)	Damage to roots leading to toppling and reduced yield	Yes*
Banana weevil (*Cosmopolites sordidus, Castniomera licus)*	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 3*x*/2*x* 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 4*x*/2*x* 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

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