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MusaNet

CGIAR RESEARCH PROGRAM ON Roots, Tubers and Bananas

CGIAR RESEARCH PROGRAM FOR Managing and Sustaining Crop Collections

Global Strategy for the Conservation and Use of *Musa* (Banana) Genetic Resources

A consultative document prepared by the Global *Musa* Genetic Resources Network (MusaNet)

2016

MusaNet is the Global Network for *Musa* Genetic Resources, coordinated by Bioversity International, with member representatives from various banana research institutes and organizations that support *Musa* research. MusaNet aims to optimize the conservation and use of *Musa* genetic resources by coordinating and strengthening such conservation and related research efforts of a worldwide network of public and private sector stakeholders. www.musanet.org

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Citation: MusaNet 2016. Global Strategy for the Conservation and Use of *Musa* Genetic Resources (B. Laliberté, compiler). Bioversity International, Montpellier, France.

The layout and design of this publication was done by Luca Pierotti.

Photo credits:
Cover photo: R. Chase/Bioversity International - CARBAP, Cameroon.
Part A: A. Vézina/Bioversity International - Nairobi, Kenya.
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Part D: J. Sardos/Bioversity International - Market in India.
Back left cover: J. Daniells/DAF, Australia.
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ISBN: 978-92-9255-050-9

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Acknowledgements

The Global Strategy for the Conservation and Use of *Musa* (Banana) Genetic Resources, coordinated by the Global *Musa* Genetic Resources Network (MusaNet), is the product of expert opinion and detailed discussions among diverse stakeholders involved in the conservation and use of *Musa* genetic resources since 2011, when MusaNet was established.

MusaNet is grateful to all those involved in developing the first Global Strategy, published in 2006. This 2016 edition builds on the first Strategy and goes beyond the focus on *ex situ* conservation of *Musa* genetic resources to also include priority actions in the areas of *in situ* and on-farm conservation, germplasm evaluation and genetic improvement.

Central to the whole process was Professor Edmond De Langhe who provided scientific leadership. His energy, commitment and dedication to this important exercise are greatly acknowledged.

Brigitte Laliberté, Advisor to MusaNet, coordinated the development of the Global Strategy in close collaboration with Edmond De Langhe and Rachel Chase. We would also like to thank all the members of the MusaNet Expert Committee for their guidance in the process, particularly Nicolas Roux and Jean-Pierre Horry, who contributed to the initial development of the document.

Grateful appreciation is expressed to Rachel Chase and Vincent Johnson for the editing of the document and for the layout professionally handled by Luca Pierotti.

We consider this Global Strategy a collaborative effort, which could not have been accomplished without the individual and group efforts of many people. We would like to thank all who participated actively in discussions and in the review of the final draft document, which was circulated to all MusaNet members and collection curators. Grateful appreciation is expressed to the lead author and co-author of each Chapter (see list in table below).

This version of the Global Strategy is therefore the fruit of the efforts of many *Musa* scientists, and conservation and use practitioners, and will be discussed regularly at international and regional and MusaNet meetings.

MusaNet is committed to overseeing the further development and monitoring of the implementation of the Global Strategy. It encourages international, regional and national public research organizations, development agencies, NGOs and the private sector to use the priorities set out herein to guide their activities and investment decisions.

The development of this Strategy was largely supported by all donors who supported *Musa* work through their contributions to the CGIAR Fund (http://www.cgiar.org/who-we-are/cgiar-fund/fund-donors-2/), and in particular to the CGIAR Research Programs (CRP) for Managing and Sustaining Crop Collections (Genebanks-CRP) and for Roots, Tubers and Banana (RTB-CRP). This is not an exhaustive list of the many funding sources for the work cited in the Strategy; therefore we thank all organizations that contributed to *Musa* projects over the years.

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Special appreciation is expressed to the following contributors of the Global Strategy, including authors and key reviewers during the meetings and consultations. MusaNet apologizes for anyone that may have inadvertently been missed.

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29. Jaroslav Dolezel (IIEB)
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31. Jeff Daniells (DEEDI)
32. Jim Lorenzen (Bill & Melinda Gates Foundation)
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60. Vida Grace Sinohin (Bioversity International)
61. Vincent Johnson (Bioversity International)
62. Xavier Perrier (CIRAD)

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MusaNet particularly thanks the following Musa germplasm collection managers for their collaboration in providing detailed information on the status of their collection through the Global Musa Survey carried out between 2012 and 2015:

1. Australia, Department of Agriculture , Forestry and Fisheries, Maroochy Research Facility, DAFF-Maroochy, Sharon Hamill
2. Australia, Department of Agriculture , Forestry and Fisheries, South Johnstone Research Facility, DAFF-South Johnstone, Jeff Daniells
3. Belgium, International Transit Centre, Bioversity International, ITC, Ines Van den houwe
4. Brazil , Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Janay Serejo and Edson Amorim
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8. China, Taiwan Banana Research Institute, TBRI, Lee Shu-Ying
9. China, Tropical and Subtropical Fruit Research Lab, TSFR, Houbin Chen and Chunxiang Xu
10. Colombia, Corporación Colombiana de Investigación Agropecuaria, CORPOICA, Alvaro Caicedo Arana
11. Colombia, Federacion nacional de Plataneros de Colombia, FEDEPLATANO, Francisco Grisales
12. Congo DRC, Faculty of Sciences, University of Kisangani, FSK, Benoît DHED'A DJAILO
13. Congo DRC, Institut National pour l'Etude et la Recherche Agronomiques, INERA, Pierre Nkongolo Muamba Dipu and Elasi Ramazani Kitima
14. Cook islands, Ministry of Agriculture, MoA, William Wigmore
15. Costa Rica, Corporación bananera Nacional, CORBANA, José Miguel González Zuñiga
16. Côte d'Ivoire, Centre National de Recherche Agronomique, CNRA, Deless Edmond Fulgence Thiemele

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19. Ethiopia, Ethiopia Institute of Agricultural Research, Melkassa Research Center, EIAR-Melkassa, Girma Kebede, and Lemma Ayele Bekete
20. Fiji, Secretariat of the Pacific Community, SPC, Valerie Saena Tuia,
21. Fiji, Sigatoka Research Station, SRS, Manoa, Iranacola, and Poasa Nauluvula
22. French Polynesia, Service du développement rural, French Polynesia national collection, SDR-FPNC, Maurice Wong
23. French Polynesia, Service du développement rural, Pacific regional field collection, SDR-PRFC, Maurice Wong
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25. Guadeloupe, Centre de coopération internationale en recherche agronomique pour le développement, CIRAD, Daniele Roques and Jean-Pierre Horry
26. India, Banana Research Station, Kerala Agricultural University, KAU, Rema Menon
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This document has been developed by *Musa* genetic resources and breeding experts. The objective of this document is to provide a framework for the efficient and effective conservation of the globally important *Musa* genetic resources and strengthening their use. This strategy document is likely to continue evolving and being updated as and when information becomes available. The views and opinions expressed here are those of the contributors and do not necessarily reflect the views and opinions of their individual institutes. In case of specific questions and/or comments, please direct them to the MusaNet Secretariat at Bioversity International (musanet.secretariat@gmail.com).

Abstract

Sweet and starchy bananas (*Musa* spp.) are crops of great importance for both the subsistence and the livelihoods of people in developing countries. Banana is also one of the most popular fruits worldwide. From their origin in Southeast Asia, bananas have spread to and diversified in the Pacific, Asia, Africa, Latin America and the Caribbean. The Pacific is also a major center of *Musa* domestication.

The nature of banana as a vegetatively-propagated, mostly polyploid and relatively sterile crop poses unique constraints for its conservation, breeding and improvement. Scientists have recently sequenced the two original parent genomes (*Musa acuminata* and *Musa balbisiana*) of most edible banana, which has led to a deeper understanding of *Musa* genetic diversity. New molecular techniques such as genotyping by sequencing (GBS), RADseq and re-sequencing are helping unlock the genetic potential of banana. These tools, when linked to phenotypic data, can ultimately help minimize losses due to pests and diseases and other stresses such as drought. Selection and breeding efforts are also directed toward improving global productivity and nutrition.

Implicit in the use of *Musa* diversity is the need to safeguard that diversity for future generations. National and regional ex situ collections, found in tropical regions across the globe, are working towards conserving and documenting their local banana diversity. More focus is also being placed on exploring in situ diversity growing on farms and in the wild. The Bioversity International Transit Centre's global collection in Belgium aims to conserve all *Musa* species and cultivars by in vitro storage or cryopreservation, and is now also investing in conserving seeds of wild *Musa* species.

The vision of the Global Strategy for the Conservation and Use of *Musa* Genetic Resources is a world in which *Musa* genetic diversity is secured, valued and used to support livelihoods of hundreds of millions of farmers through sustainable production and improved food and nutrition security. It includes actions that aim to i) assess *Musa* genetic diversity and correctly identify and fill gaps in the diversity, ii) conserve the entire *Musa* gene pool in perpetuity in ex situ collections, in situ in the wild and on farms, iii) maximize the use of genetic diversity through comprehensive characterization of the accessions and their evaluation, iv) apply genomics tools to banana to better support breeding and v) document the germplasm and make the information easily accessible to users.

This Strategy was developed following consultation with a wide range of stakeholders from the global *Musa* research community, especially linking with MusaNet. It covers numerous topics dealing with *Musa* genetic resources, with the 12 chapters divided into five main sections: Introduction, Plant Diversity, Identification, and Management and Use. It concludes with a section summarizing actions to be taken, and then a series of annexes of important complementary information. Throughout the document, each chapter introduces current status (where we are now) and then proposes where and how the *Musa* community would like to proceed (where we want to go, and how we will get there).

The proposed actions will be implemented through the Global *Musa* Genetic Resources Network, MusaNet. With its five thematic groups (Conservation, Diversity, Evaluation, Genomics and Information) and representatives from all major banana-producing regions, MusaNet aims to ensure the long-term conservation of *Musa* on a cooperative basis, and facilitate the increased utilization of *Musa* genetic resources globally.

Introduction to the Global *Musa* Strategy

MUSANET

This 2016 Global Strategy for the Conservation and Use of *Musa* Genetic Resources has been developed by *Musa* genetic resources and breeding experts within the framework of the Global *Musa* Genetic Resources Network - MusaNet.

MusaNet is the Global Network for *Musa* Genetic Resources, coordinated by Bioversity International with member representatives from various banana research institutes and organizations that support *Musa* research. MusaNet aims to optimize the conservation and use of *Musa* genetic resources by coordinating and strengthening the conservation and related research efforts of a worldwide network of public and private sector stakeholders. www.musanet.org

MusaNet is committed to overseeing the further development and monitoring of the implementation of a Global Strategy for the Conservation and Use of *Musa* Genetic Resources (the Global Strategy hereafter). This version of the Global Strategy is therefore the fruit of the efforts of many *Musa* scientists, and conservation and use practitioners, and will be discussed regularly at regional and MusaNet Expert Committee meetings. MusaNet encourages international, regional and national public research organizations, development agencies, NGOs and the private sector to use the priorities set out herein to guide their activities and investment decisions.

The Global Strategy is intended to be used as a roadmap for the *Musa* genetic resources community and proposes a collaborative framework for proposed activities on the conservation and use of *Musa* genetic resources.

DEVELOPMENT AND STRUCTURE OF THE STRATEGY

A first Global Conservation Strategy for *Musa* was first developed in 2004-2006, in consultation with a large number of individuals (INIBAP 2006). In 2011, the Expert Group that established MusaNet recommended reviewing and updating the 2006 Global Strategy. A new survey of the status of *ex situ* collections was then conducted during 2012-15 (see Annex D - MusaNet Global Survey of ex situ collections – 2012-2015). Over 50 collections participated in updating the information on the status of *Musa* collections worldwide (see Annex D – Table D.1 *Collections that replied to the Global Musa Survey*). During the period from 2012-2013, each of the five MusaNet Thematic Groups[1] developed a workplan with priority actions.

The document is divided into five main sections consisting of 12 chapters. This first section introduces the context of *Musa* genetic resources conservation and use, followed by the main body of four sections on the diversity, identification, management and use of *Musa* germplasm. The document concludes with a section summarizing actions to be taken, and then a series of annexes of important complementary information. Throughout the document, each section introduces current status (*where we are now*) and then proposes where and how the *Musa* community would like to proceed (*where we want to go,* and *how we will get there*).

After introducing the Strategy structure, the introduction offers a general background for global banana production. It then outlines breeding programmes and genetic improvement, key advances in research and development, and major constraints and risks affecting the current global system. It goes on to articulate the Strategy objectives and expected outputs, and describe networking and partnerships that are critical for both developing and implementing the Strategy.

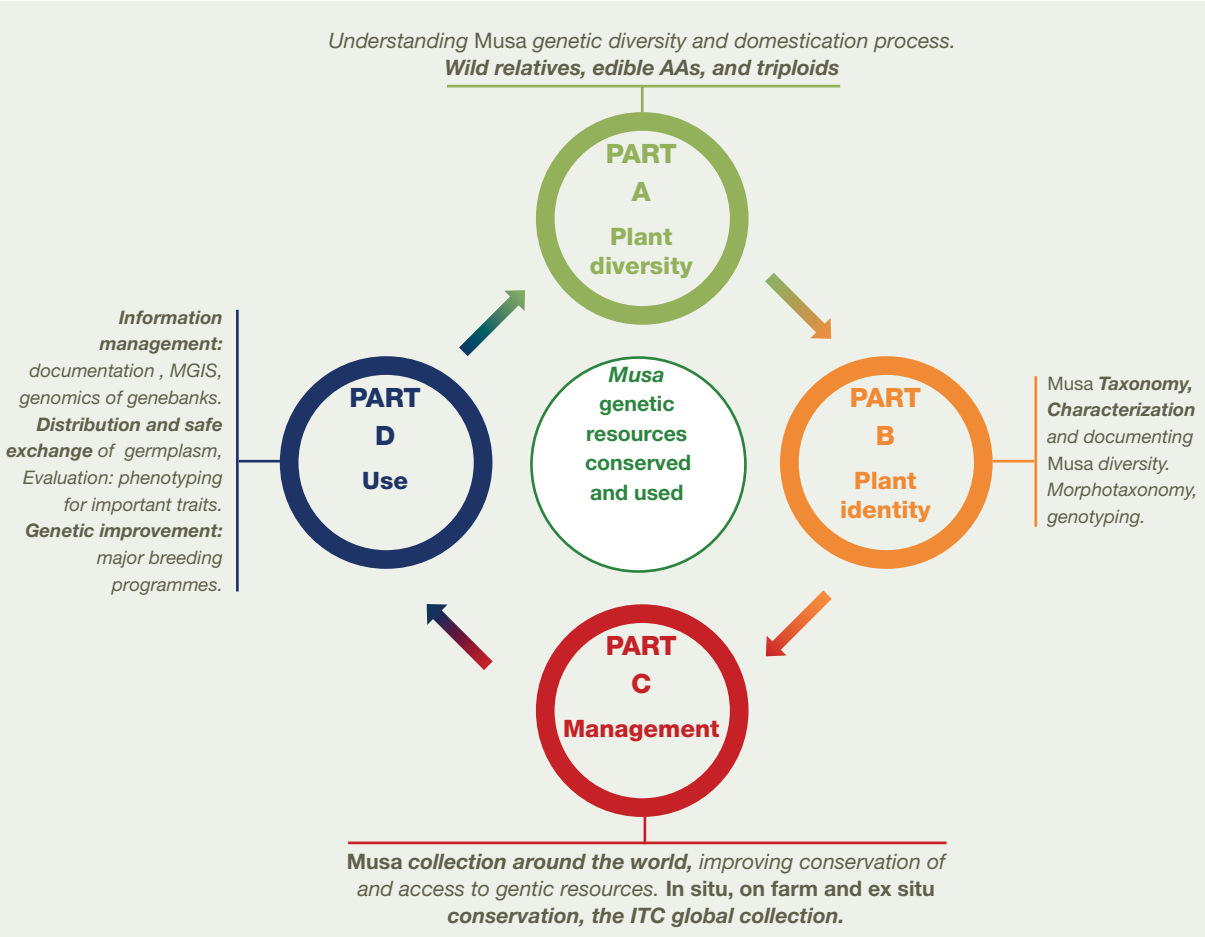
1 The five MusaNet thematic groups are diversity; conservation; evaluation; information and genomics.

A review of the current knowledge on banana diversity (Part A – Plant Diversity) reveals several gaps in the identification of many wild species and cultivars. The gaps are tackled by rigorous instruments for reliable identification of the taxa (Part B – Identification). The progressively acquired taxonomic knowledge is the major input for efficient management of the germplasm, via appropriate collections of correctly characterized taxa (Part C - Management). Although the exact identity of plant material is an evident prerequisite for all users, data about both agronomic performance, such as productivity and resistance to pests and diseases, and quality, such as fruit flavour and processing characteristics are critical in the selection of desired cultivars. The Strategy should thus meet these requirements of correct identity and of agronomic and quality values for any taxon (Part D - Use).

This document aims to provide a clear framework and roadmap to be used by the *Musa* community for the efficient and effective conservation of the globally important collections of *Musa* and strengthening the utilization of the genetic resources. It includes recommendations and priorities indicated in several consultation processes following the 2006 Strategy and particularly the expertise and key groups represented including the Regional Research Networks such as BAPNET[2] , BARNESA[3] , InnovatePlantain, MusaLAC[4], and global networks such as ProMusa.

Figure 1.1 reflects the desired optimal situation as a logical flow of operations. The Strategy is cyclic in nature, because knowledge exchanged among its parts should help in adjusting further operations.

Figure 1.1. *Diagram visualising the components of the Global Strategy.*



2 Banana Asia-Pacific Network

3 Banana Research Network for Eastern and Southern Africa

4 Plantain and Banana Research and Development Network for Latin America and the Caribbean

BANANA AND PLANTAIN PRODUCTION WORLDWIDE

In 2013, more than 145 million metric tonnes of bananas and plantains were produced in over 130 countries on more than 11 million hectares of land. The top producers are India, with around 27 million metric tonnes (19% of total production), and China with 12 million metric tonnes (8% of total production). Uganda is the third-largest producer with around 9.5 million metric tonnes (cooking and beer bananas – making up 7% of total production), followed by the Philippines (8.6 million metric tonnes, 6% of production) and Brazil (6.9 million metric tonnes, 5 % of total production). (FAO 2013).

WHY IS THE PRODUCTION IN DANGER?

The fragility of the banana

The productivity of cultivated banana varieties (cultivars) is more exposed to environmental accidents than that of most other food crops.

Banana cultivars, being vegetatively propagated, are clones of which all the individual plants possess exactly the same genotype. Any upcoming serious stress in a region potentially menaces all the plants of the cultivar wherever it is grown in the world. Moreover, somatic mutations occurred during the centuries-long propagation of the presumed original clone, generating wide phenotypic variations with apparently still the same basic genotype, called a ‘subgroup’. This means that an entire subgroup suffers from the stress.

Nearly half of global banana production relies on a limited number of genetic combinations represented by a single clone (Cavendish), with another major clonal group, Plantains, representing a further 16%. Such a lack of cultivated genetic diversity leaves the crop highly vulnerable to pests and diseases and other risks (Calberto et al. 2015).

Banana plants need nine months to two years from planting to produce the first fruit crop, a longer time than for most other vegetatively propagated food crops. Susceptible cultivars are sometimes replaced by more resistant ones, but this is a cumbersome and lengthy process. In several regions, it obliges traditional consumers to accept fruits with different qualities and flavour, and farmers and merchants to change harvesting, packaging, ripening and processing procedures. Sadly, consumers often accept such changes only after a disease has almost completely erased the popular subgroup, as in the case of the Pacific islands with the Maoli-Popoulu subgroup - a very poor alternative.

Finally, on account of their often triploid or hybrid nature, many banana cultivars are sterile so that even occasional seed production in a banana field does not occur, in contrast to several other vegetatively propagated food crops - such as cassava, potato, sweet potato - where spontaneous seedlings can broaden the genotype, thus creating new genetic populations and offering chances of finding superior, more resilient individuals.

Consequently, the concept of selection among the existing diversity that would combine all the required criteria is rarely an efficient mode for productivity improvement. Genetic improvement, or the construction of new germplasm (artificial hybrids), is the only viable alternative.

To certain degree, each of the above deficiencies can be found in other food crops, but it is their overwhelming accumulation in a single crop that makes the banana extraordinary fragile.

GENETIC IMPROVEMENT OF THE BANANA

In the 20th century, the large fields of the dessert cultivar Gros Michel became devastated by Fusarium wilt, caused by a *Fusarium oxysporum* var. *cubense* (*Foc*) race 1. Another subgroup, the Cavendish bananas, appeared to be resistant to that race and to produce fruits with the exquisite taste of Gros Michel. Cavendish has since become the worldwide alternative in the dessert banana market. Meanwhile the genetic improvement of Gros Michel was started by British breeders in Trinidad. One of the motivations was that a new *Foc* race could in the future equally wipe out the Cavendish fields, a concern which has indeed become a reality in recent years with the invasion by *Foc* race 4, beginning in Taiwan and spreading now over Southeast Asia and into the African continent. But until now, no satisfactory new hybrid could be selected for acceptable replacement on the market, neither for Gros Michel nor more recently for Cavendish.

However, during the second part of the 20th century, several other diseases and pests began to attack the traditionally cultivated starchy bananas, in many regions, mainly due to the rapidly growing intensity of human contacts. Abiotic stresses became also important in some regions due to climate change effects. This prompted several institutes to embark on genetic improvement of the traditional subgroups.

Because the local market for food bananas is much more relaxed on the quality of the fruit compared to the commercial dessert market with its strict criteria, it was expected that selected new hybrids would relatively soon meet various stress problems.

However, based on sources of biotic and abiotic stress-resistance from wild and edible diploid genotypes, these hybrids have yet to meet even the more flexible criteria, such as widely-acceptable fruit-pulp quality. The reason appears to be that only a fraction of the genetic diversity in diploid *Musa* is being used, leading to limited sources of desired characteristics. Yet variation among wild and edible *Musa* species offers a wide spectrum of fruit and bunch qualities.

Taxonomists, breeders and researchers attribute under-utilization of such material to inadequate information. Many *Musa* collections have not been systematically documented; only limited characterization/identification and evaluation data are available, and information may be scattered among several institutes. Limited information is preventing users from identifying the most appropriate accessions.

The recent impressive progress in the elucidation of the molecular basis of plant diversity has brought the hope that it will considerably boost the breeding efforts. Indeed and for example, the Next Generation Sequencing (NGS) of the *Musa* DNA should allow characterization of crops that would be further used to tag germplasm and to locate particular DNA sequences of interest, including those controlling some important traits. Indeed whole genome NGS approaches are currently adopted for *Musa*, such as Genotyping by Sequencing (GBS) and RADseq in addition to re-sequencing of wild diploids' genomes. Such developments in molecular biology should have an important impact on the role of genebanks and the use of genetic resources. However, it appears that the potential contribution of genomics to the improvement of the banana is only starting to be understood (since the sequencing of the whole *Musa A* genome in 2012), hence as yet weakly integrated in the *Musa* improvement programs, in contrast with other crops such as wheat and maize.

CONTAINING THE STRESSES AS A PROVISIONAL ALTERNATIVE TO GENETIC IMPROVEMENT

With the current and rather lasting rarity or absence of satisfactory resistant new hybrids, farmers are challenged to protect their fields as much as possible from invading destructive biotic agents and adverse abiotic events. For most farmers, pesticide applications are too costly for combating pests and diseases. Fortunately, some diseases such as the Bacterial Wilt, and pests such as nematodes and borers can be contained using appropriate and proven integrated pest management (IPM) practices.

However, results in one area are not always applicable to other areas or regions, partly due to confusion in the identity of the concerned cultivars. Lack of correct cultivar classification and abundant synonyms of cultivars in many regions has led to frequently misunderstood cultivar information, resulting in inadequate practices.

While interested organisations rely on local scientific expertise, they are frequently not able to propose more promising cultivars. Numerous national collections are functioning sub-optimally, particularly those which are hosted by poorly-resourced organizations. In several cases accessions are diseased and under threat, germplasm exchange mechanisms are inadequate and the user community is not as well served as it might be. In most collections, there are no quality control mechanisms to ensure that collections are fully accessible and material is safely exchanged. There is insufficient skilled staff to meet the long-term conservation needs. Curators are often working in isolation with little training. Most of the collections require additional human resources for the general collection management and particularly technical support in classification.

Thus banana production remains precarious despite many serious, but rather disparate efforts to enhance productivity. The situation is partly due to the fragile nature of the plant and suboptimal coordination of efforts in both genetic improvement and optimizing field maintenance.

This Global Strategy document attempts to meet the constraints in the exploitation of the *Musa* diversity by providing the information that should facilitate regular consultation on a global scale, including but not limited to the response to various stresses on the banana crop.

TARGET AUDIENCE FOR THE GLOBAL STRATEGY

The aim of this Global Strategy is to provide a framework for the efficient and effective conservation of globally important *Musa* genetic resources and strengthening their use. The Strategy can stimulate partnerships that increase the impact of research and adoption of technological innovations. MusaNet therefore encourages international, regional and national public research organizations, development agencies, NGOs and the private sector to use the priorities set out herein to guide their activities and investment decisions. The Global Strategy provides a clear framework to secure funding for the most urgent needs, to ensure that *Musa* diversity is conserved and used, and provides direct benefits to the hundreds of millions of small-scale banana farmers around the world.

STRATEGY OBJECTIVES AND EXPECTED OUTPUTS

The conservation and use of a wide range of genetic diversity of *Musa* for future breeding depend on effective collaboration and the identification of clear common objectives with an action plan where all stakeholders play a role.

The VISION of the Global Strategy is:

- A world in which *Musa* genetic diversity is **secured, valued and used** to support livelihoods of hundreds of millions of farmers through **sustainable production and improved food and nutrition security**.

The Strategy builds upon existing strengths in the national, regional and global collections by bringing people together to optimize the effort to conserve, add value and promote the use and safe distribution of a wide range of *Musa* genetic diversity as the foundation for further breeding and in some cases, direct use by farmers.

The EXPECTED OUTPUTS are:

- The *Musa* genetic diversity is assessed and comprehensively characterized, the taxonomy is harmonized and gaps in diversity are identified and filled in *ex situ* collections.

- The entire *Musa* gene pool is conserved in perpetuity by a network of well-managed and rationalized collections.
- The global system for the safe exchange of *Musa* germplasm is strengthened through appropriate diagnostics, screening, quarantine and exchange protocols etc.
- The use of *Musa* genetic diversity is maximized through user-oriented germplasm evaluation.
- Information on all aspects of *Musa* germplasm is well documented and easily accessible to all users in user-friendly and efficient systems.
- Sufficient numbers of stakeholders are trained, and able to apply their training on the subjects of *Musa* Diversity, Conservation, Information, Evaluation and Genomics.

Furthermore, the conservation of *Musa* diversity contributes to the United Nation's Sustainable Development Goal (SDG) targets for 2030, namely Target 15 (*Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss*), with implications for Target 2 (*End hunger, achieve food security and improved nutrition and promote sustainable agriculture*) (https://sustainabledevelopment.un.org).

PARTNERSHIPS AND NETWORKING

The Global Strategy offers opportunities for greater and stronger collaboration in all aspects of conservation and use of *Musa* genetic resources, spelt out in the Section 2 – *Where we want to go* and Section 3 – *How we will get there* of each chapter.

Global collaboration for an efficient and effective system requires the full support and participation from all key partners. At the heart of the Global Strategy are the institutes managing *Musa* diversity and the custodians and users of these resources.

There is a need to create a vibrant community sharing more and better information and knowledge, working together on agreed priorities. The main incentives for participants in developing and implementing the Global Strategy are: 1) greater access to information and germplasm, 2) acknowledgment of data sources particularly in publications, 3) benefits from training, 4) opportunities to participate in research projects and, 5) developing and sharing a common methodology and standards, thereby facilitating collaboration.

There is a need for more awareness and discussions between partners including donors, and to stimulate interest in genetic resources collecting, characterization, conservation, evaluation and utilization.

A number of networks and global initiatives are directly supporting the implementation of the Global Strategy and are described in Annex A, including: MusaNet, the Regional Banana Research and Development Networks, ProMusa, the CGIAR research program – Roots, Tubers and Bananas (CRP-RTB), CRP-Genebanks, the International Treaty for Plant genetic Resources for Food and Agriculture (ITPGRFA), the Global Crop Diversity Trust (GCDT) and the National, Regional and International Institutes managing *Musa* genetic diversity.

A collaborative platform such as MusaNet is the ideal instrument for coordinating the implementation of a strong and focused Global Strategy with clearly defined goals and roles, including those complementary roles of national collections, regional collections and the International Transit Centre (ITC).

PART A

DIVERSITY

Part A describes the extent of *Musa* diversity in the next three chapters: Chapter 1 covers *wild relatives and domestication* (including exploration and collecting priorities); Chapter 2 covers the *edible diploids* and Chapter 3, the *triploids*.

CHAPTER 1.
WILD RELATIVES AND DOMESTICATION

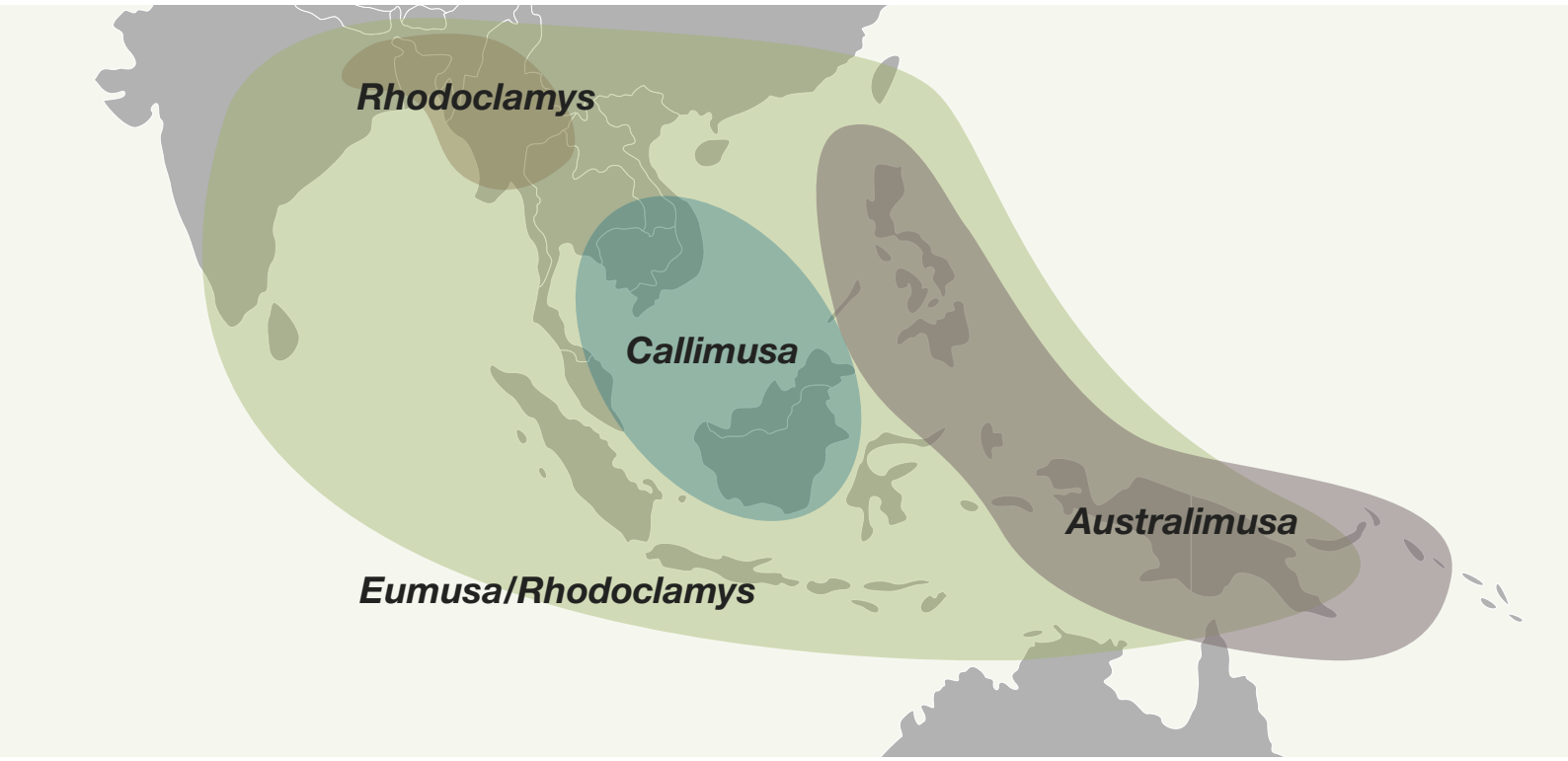
SECTION 1.1 WILD RELATIVES AND DOMESTICATION - WHERE WE ARE NOW

1.1.1 The ancestors of the edible bananas

All edible bananas and plantains stem from the genus *Musa*, which represents a group of approximately 70 forest-dwelling species, distributed between India and the Pacific, as far north as Nepal and extending to the northern tip of Australia.

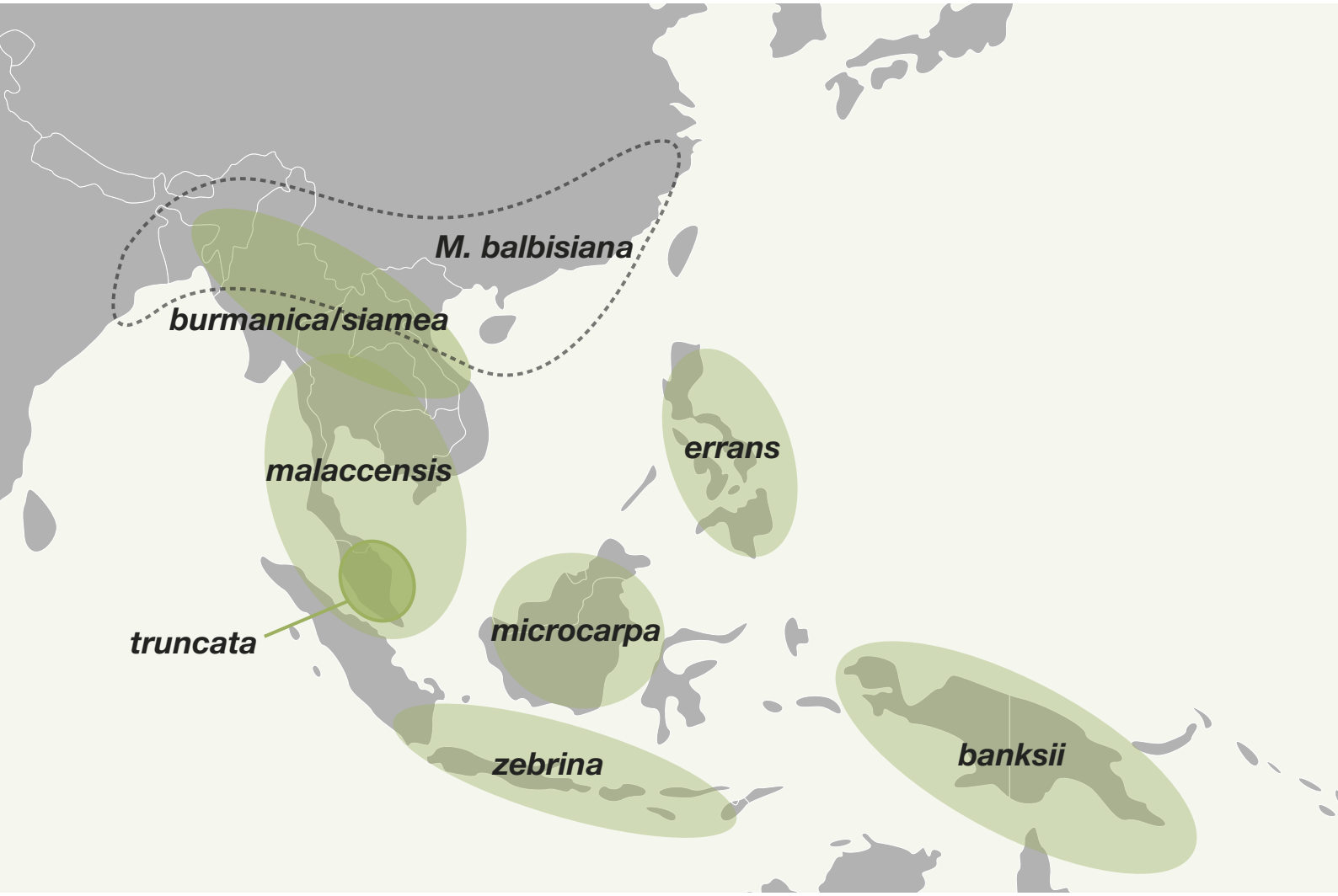
The genus belongs to the family Musaceae, which also comprises some seven species of Ensete and possibly a third, monospecific genus, Musella, which is related to *Musa*. The *Musa* genus is divided into four sections: Eumusa, Rhodochlamys, Callimusa and Australimusa. The geographic origins of the sections are shown in Figure 1.2.

Figure 1.2. *Distribution of the four sections of the genus Musa. (Source: De Langhe et al. 2011, Fig 1, slightly modified).*



All species are diploid, with a basic chromosome number n=11 (Eumusa, Rhodochlamys) or n=10 (Australimusa, Callimusa), with the uncertain placement of *M. beccarii* n=9 and *M. ingens* n=7. *M. acuminata* displays a large variation, mainly in inflorescences, and is divided into several subspecies (see Figure 1.3). The species contributes what is called the 'A' genome to the edible bananas, and its subspecies *banksii* is believed to be at the origin of the domestication process.

Figure 1.3. *Natural distribution of the M. acuminata subspecies and of the species M. balbisiana (Source: Perrier et al, 2011).*



The much less variable *M. balbisiana* contributed the 'B' genome to several banana cultivar subgroups and all plantains.

Banana cultivars commonly contain one or more A or B genomes, or both, as diploids (AA and AB), as triploids (AAA, AAB, ABB), and as some rare tetraploids (AAAA, AAAB etc.).

A third species, *M. schizocarpa* has contributed to the formation of diploid cultivars (AS) and a few triploid hybrids in New Guinea. Wild and cultivated diversity of *Musa* is at its richest in the Asia and Pacific region.

An additional group of edible bananas, known as Fe'i bananas, are confined to the Pacific. Their genetic origin is obscure, but taxonomic studies suggest ancestral links either with the wild species *M. maclayi* or *M. lolodensis*, which belong to the Section Australimusa.

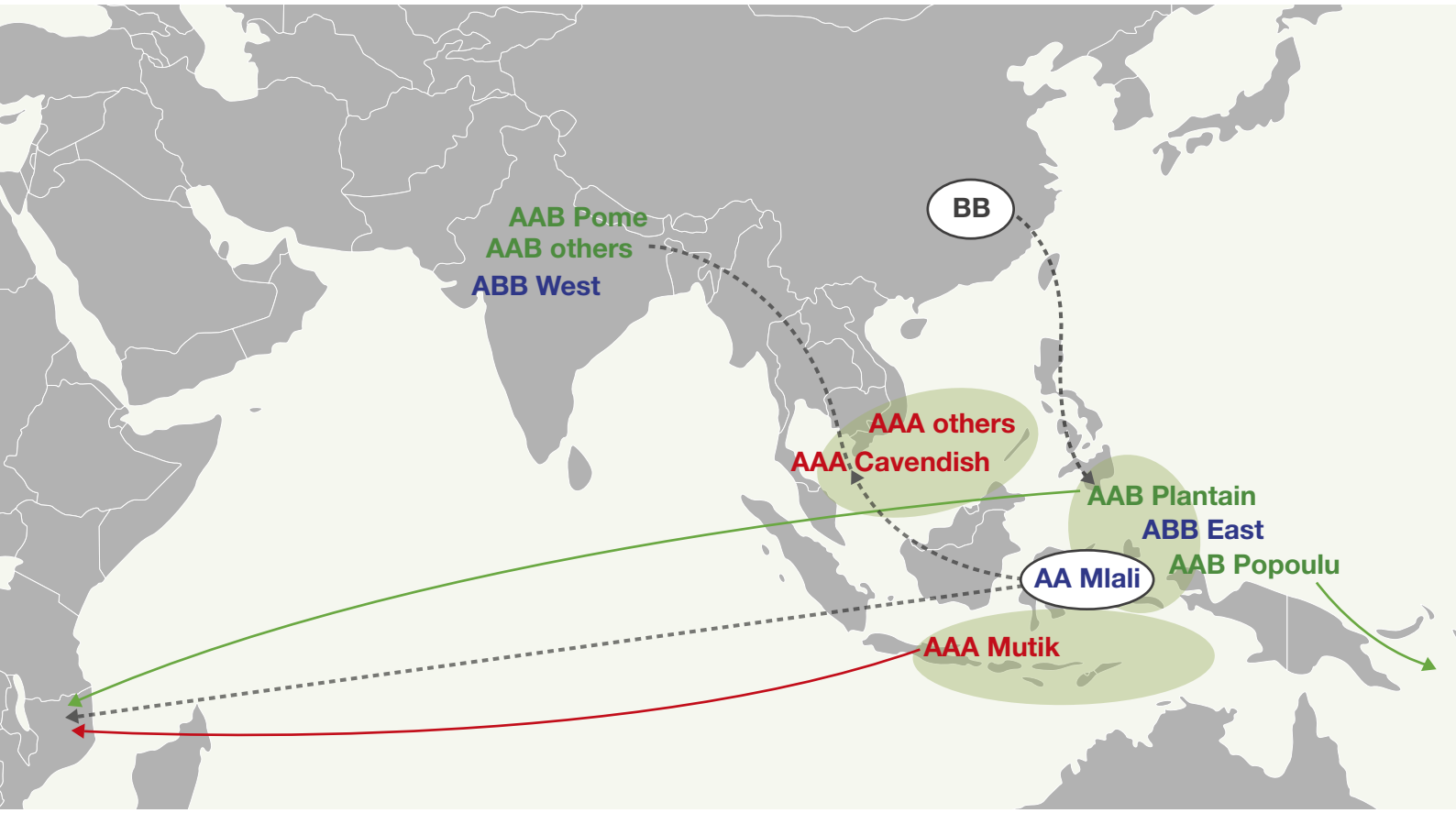
1.1.2 From wild to edible

When, where and how did the edible character arise, namely, a fruit being acceptably non-seeded and with sufficient pulp? The key point of banana domestication for fruit edibility is the establishment of spontaneous parthenocarpy associating abundant pulp development and partial or total sterility. These two characters are linked by a parallel selection but their evolutionary process is not necessarily the same. The simplest explanation is a temporal sequence of partial parthenocarpy, followed by inter-subspecies hybridization in the *M. acuminata* area and a resulting partial sterility. The evolution to partial parthenocarpy can still be observed in at least three regions: in Ethiopia among *Ensete* populations, in northern India among populations of *M. balbisiana*, and in the Pacific among the Fe'i bananas. In the three cases, the plants are vegetatively propagated and produce more or less soft, edible seeds surrounded by pulp.

Hence it is assumed that the same process initially took place about 8,000 years ago with the *M. acuminata* subspecies *banksii* in the New Guinea region, thus producing the first edible AA diploids. Human interaction would subsequently bring these edible AA in contact with other (wild) *acuminata* subspecies. Spontaneous crosses would then have generated partial sterility in new edible AA, principally in the Indonesian and Philippines regions. Millennia-long cloning of the attractive hybrids eventually led to the large spectrum of current edible AA, producing fully parthenocarpic and seedless fruits, but with a moderate maintenance of viable pollen production in some cultivars.

The last step of domestication is the emergence of triploid cultivars that constitute the majority of popular cultivars currently grown in the tropical world. Triploid cultivars are a direct consequence of the perturbed fertility of edible diploids, where diplogametes combined with regular n-gametes allowed the emergence of triploid genotypes, monospecific (AAA) or interspecific (AAB, ABB). Figure 1.4 shows the migrations of the triploid subgroups.

Figure 1.4. *Origins and migrations of the main triploid subgroups. (Source: De Langhe et al. 2009).*



The plain arrows in Figure 1.4 indicate long-term prehistoric migrations of triploid cultivars to Africa and Pacific islands. Grey dotted arrows indicate: (1) the migrations of Mlali AAcv subgroup, which is not found in island Southeast Asia today, to mainland South East Asia where it contributed to AAA Cavendish then to India where it met *M. balbisiana* to give AAB Pome; (2) migrations of the Mlali subgroup to the East African coast. The black dotted arrows indicate the route of *M. balbisiana* from South China to New Guinea over Taiwan and the Philippines, as carried by ancestors of the Austronesian speakers or perhaps sooner (Perrier et al. 2011).

SECTION 1.2 WILD RELATIVES AND DOMESTICATION - WHERE WE WANT TO GO

By virtue of their natural variation, wild *Musa* species are an essential source of desired traits in genetic improvement programmes. The parents *M. acuminata* and *M. balbisiana* are used in breeding schemes, both directly to produce improved cultivars and indirectly to improve parental genetic stocks. Other species are potential sources of traits but complicate the breeding schemes due to their genetic distance.

Host-pathogen interactions differ among the subspecies of *M. acuminata*. For example the subspecies (ssp) *burmannica* is resistant to black leaf streak disease while the ssp. *banksii* is rather susceptible. But variations in reaction to diseases are supposed to exist even within the ssp. *banksii*, and similar variations may exist in the other subspecies. Subspecies differ also in their habitat, with e.g. ssp. *truncata* and *burmannica-siamea* typical for higher altitudes. But again, specimens of such subspecies have been observed at lower altitudes. Being fully inter-fertile and naturally out-crossing, it is most likely that *M. acuminata* subspecies consist of populations of genetically close individuals. *M. balbisiana* cultivars are vigorous and tolerant to several diseases, but may differ in the degree of these qualities, and this has not been sufficiently studied. Moreover, while new wild species and cultivars continue to be described, they are inadequately represented in *ex situ* collections.

In summary, the variation among both species and subspecies is still poorly known and there is a need to organise the progressive collection of the complete genetic diversity of wild species. However, some wild banana accessions are threatened in *ex situ* collections because they may not be grown in optimum conditions similar to their specific habitat. The idea of satellite collections in the appropriate environments is interesting but practically hardly applicable. The best solution is *in situ* conservation of such taxa, with due recording of locations with GPS positions. This calls for a coordinated effort involving national parks and reserves playing a crucial role (see Chapter 8 - *in situ and on farm conservation*).

SECTION 1.3 WILD RELATIVES AND DOMESTICATION - HOW WE WILL GET THERE

1.3.1 Exploration and Collecting Priorities

The following priority areas for collecting *Musa* are also discussed in detail in Section 4.3.1 – *Exploration and Collecting Missions*.

1.3.1.1 Indonesia

MusaNet, through funding from the CGIAR Research Program on Roots, Tubers and Bananas (CRP-RTB) and the Belgian Government, coordinated two collecting missions in Eastern Indonesia in October 2012 and in February-March 2013 where both wild and edible *Musa* had previously been only minimally explored. The missions were led by scientists of the Indonesian Tropical Fruit Research Institute (ITFRI), the Indonesian Centre for Horticulture Research and Development (ICHORD) and the Department of Agriculture, Fisheries and Forestry (DAFF, Queensland, Australia).The first mission took place in North Sulawesi at the area of Gunung Ambang and Bogani Nani National Park and collected 29 accessions including 11 wild species (*M. acuminata* and *M. lolodensis*) and 18 cultivars. The second mission took place in Ambon and Seram Islands (Masohi, Manusela, Wahai and Kairatu) and collected 21 accessions including 11 wild *M. acuminata* and 10

cultivars. Fresh cigar leaf samples were sent to the *Musa* Genotyping Centre (MGC) in the Czech Republic for ploidy analysis and genotyping. The description of the found taxa complemented with molecular marking (simple sequence repeats-SSRs) showed the results to be congruent with field observations. Regarding wild resources, the rich harvest includes several unrecorded cultivars of *M. acuminata* – revealing a much more complex subspecies situation, and of *M. lolodensis* (section Australimusa). Mission members stressed that the brief exploration was insufficient to detect the diversity in sufficient detail and that efforts to that end should best be organised at national/provincial level. The next step would be to co-organize more local-level detailed explorations.

1.3.1.2 Myanmar

With the exception of the *M. acuminata* ssp *burmannica*, no wild taxa have been duly recorded in this large country. Yet, in its northern region several species are supposed to be sympatric, with local variations or even intermediary taxa as the likely consequence. It may also offer the taxonomic link between *M. acuminata* and *M. flaviflora* (known only from identification in extreme North East India, and currently absent in the field collections). The indications of the political situation becoming settled seem to open the prospects for planning an organised exploration in the near future.

1.3.1.3 Indonesian New Guinea

Results of the recent Triangle exploration in Eastern Indonesia indicate that cultivars of *M. acuminata*, found in Maluku and not resembling the subspecies *banksii* or *microcarpa*, exist in Irian Jaya as well. They may have played a role in the generation of edible AA. Because the knowledge of wild taxa in Indonesian New Guinea relies on incidental observations in rather coastal areas, a systematic exploration mission is needed to assess the ill-defined diversity.

1.3.1.4 East Africa

Surprisingly, seedy *M. acuminata* populations had been found already in the 1940s on the island of Pemba. They could not be firmly classified and duplicates did not survive at the old collection in Trinidad. Recently, similar populations were detected in Madagascar which most probably represent naturalised *M. acuminata* remnants of ancient introduction(s). A more systematic investigation of the populations in Pemba and Madagascar is thus needed.

CHAPTER 2.
EDIBLE DIPLOIDS

SECTION 2.1 EDIBLE DIPLOIDS - WHERE WE ARE NOW

Edible diploids are of fundamental importance for genetic improvement. Many of them still produce more or less fertile pollen and/or when artificially pollinated, can produce hybrids with parthenocarpic fruits. While the diversity of edible diploids is subject to the same constraints as that of the triploids (see Section 3.1.2), these diploid derivatives of wild taxa have the potential to introduce the desired traits (e.g. abiotic and biotic stress resistance in wild sources) into new hybrids as well as qualities linked to edibility and agronomic performance. Breeding schemes are using this advantage in different combinations for the eventual production of improved triploids.

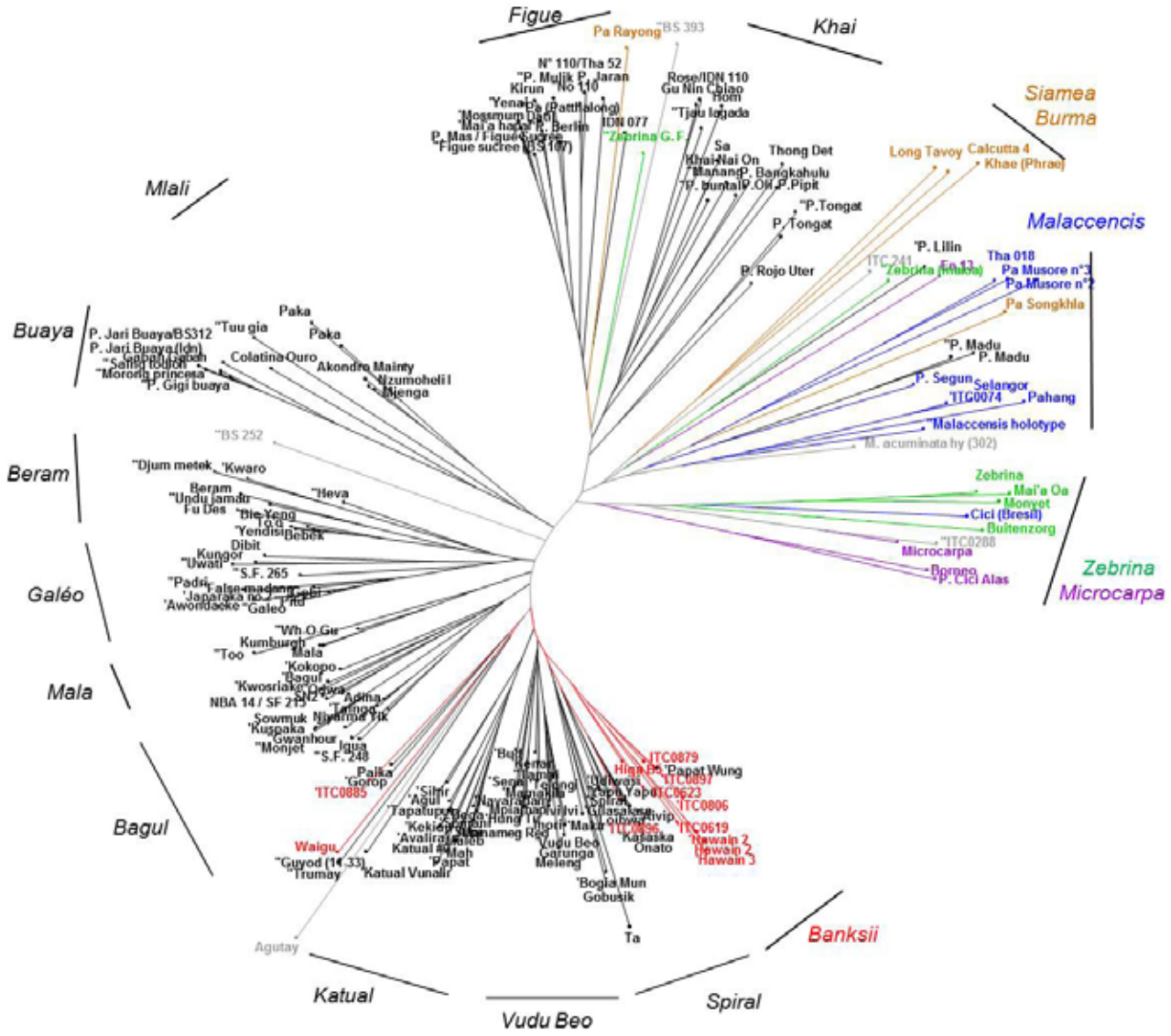
2.1.1 Edible AA

The number of existing edible *M. acuminata* diploids (AAs) cultivars is uncertain, but the great majority has apparently been collected and conserved. Papua New Guinea is the only place where AA cultivars are truly abundant (Ploetz et al. 2007; Arnaud and Horry 1997). More than 100 edible AAs have been collected in Papua New Guinea alone and produce starchy fruits. A large assembly of edible AAs is grown in East Africa and coastal islands (Comoros, Ethiopia, Kenya, Mayotte Madagascar and Tanzania,). The rest, cultivated in Southeast Asia (Indonesia, Philippines, mainland Southeast Asia, and a few in South India), have generally a sweet taste and are consumed as desert bananas.

While most of these edible AAs have at least one A-genome of the New Guinean ssp. *banksii*, the molecular evidence shows that the 'sweet' pulp of the 'western' AA cultivar derives from Southeast Asian *acuminata* subspecies, several of which produce minimal, sweet-tasting pulp (see also Section 1.1.2). The cultivar Sucrier (Pisang Mas) has a delicate sweet pulp, making it internationally popular. Many cultivars such as Pisang Jari Buaya and Pisang Lilin are used in breeding schemes.

Consequently, edible AAs offer a wide spectrum of genotypes among which the breeding programmes can select the most appropriate cultivars. Unfortunately, these many diverse edible AAs have as yet not been completely classified. The results of genetic marker techniques (SSR, DArT) on almost all collected edible AAs clearly reveal several clusters (see Figure 2.1). However, the morphological counterpart of such a cluster-concept has yet to be systematically assessed.

Figure 2.1. *Clusters of edible AAs revealed by SSR. The clusters in colour are potential wild ancestors. Source CIRAD, adapted from Perrier et al. 2011.*



2.1.2 Other edible diploids

There are surprisingly few other edible diploids. A few variants of two AB hybrids (Kunnan and Ney Poovan) are grown in South India, and one AB (Auko) was recorded in New Guinea. However, the latter shows all the characteristics of *M. balbisiana* and genetic marker research should assess its real genome composition. Research is also required for the solution of a lasting problem about supposed edible BB diploids, apparently found in mainland Southeast Asia and the Philippines: their diploidy needs be duly confirmed and the type of parthenocarpy be studied (oral reports attest of pulp with soft, comestible seeds). The Indian ABs are used in the CIRAD breeding programme (see Section 12.1.4.2 - Breeding at CIRAD).

SECTION 2.2 EDIBLE DIPLOIDS - WHERE WE WANT TO GO

2.2.1 The Taxonomy of the edible diploid diversity needs to be settled

The Taxonomic Advisory Group (TAG) 2008 meeting proposed initiating a project to identify edible AA clusters using all available characterization data. The collections with the most AAs are: the collection at the Indonesian Tropical Fruit Research Institute (ITFRI), the Laloki collection in Papua New Guinea, the Fruit and Vegetable Research Institute (FAVRI) collection in Vietnam, the collection in Malaysia at the Malaysian Agriculture and Development Institute (MARDI), and at the Bureau of Plant Industries (BPI) in the Philippines. Data will be compiled and analysed and compared with the results of molecular characterization.

Edible AAs and even 'wild' *M. acuminata* populations have been incidentally discovered in Coastal East Africa, Madagascar and islands in Indian Ocean. In and around the Comoros (and even on the African continent) edible AAs have been collected and genetically studied. Some of them were classified as the subgroup 'Mlali' which turns out to be the major ancestor of the commercial AAA 'Cavendish' subgroup, and probably Gros Michel as well (Raboin et al. 2005). All are unique in the sense that no similar cultivars are known in the region from where they supposedly originated (Indonesia, Philippines, and New Guinea). Genetic links with the seedy *M. acuminatas* on coastal East African islands cannot be excluded.

The even more complex situation of presumed edible BB found in mainland Southeast Asia and the Philippines must be elucidated. If these edible BB are confirmed, they would be a most practical building block for the construction of new AAB and ABB hybrids.

SECTION 2.3 EDIBLE DIPLOIDS - HOW WE WILL GET THERE

Due to the lack of an orderly morphological classification of the numerous edible AAs as mentioned earlier, the strategy is (1) to use standard and reliable molecular marker techniques for revealing all possible genetic differences (including clusters of same genotypes), and subsequently (2) to compare this differentiation with the observed cultivars in order to sort out subgroups and possible singletons.

1. Molecular marking of the edible AA in general

An ongoing RTB-supported Genotyping by Sequencing (GBS) project is focused on the edible AA accessions as well as a spectrum of wild AA taxa and has produced many SNPs. The lengthy work to identify and categorize the allele polymorphisms and to link these to cultivar descriptor-states will require sufficient partners and financial support. GBS will thus significantly improve the results from previous SSR and DArT techniques.

2. Edible AA in Coastal East Africa, Madagascar, and islands in Indian Ocean

The DNA from leaf samples of the collected specimens will be analysed via advanced molecular marker techniques. Particularly important is the clarification of the 'Mlali' subgroup, which contains widely different morphotypes, while these have been previously clustered together via former analyses using molecular techniques such as SSR.

3. Edible BB

Of any presumed edible BB accession, leaf samples should be sent to the MGC for ploidy verification. Duplicates of confirmed BB diploids should then be sent to ITC for conservation but also to facilitate all further relevant research.

CHAPTER 3. TRIPLOIDS

SECTION 3.1 TRIPLOIDS - WHERE WE ARE NOW

3.1.1 Triploid Bananas as we know them

Triploids are today cultivated throughout the world. They are classified according to their genome constitution as AAA, AAB, ABB, which are called 'groups'. Each group consists of a number of subgroups with different plant appearances and different genetically proven genotypes. A subgroup is defined as representing the cultivars generated from a common ancestor as caused by variations during centuries-long cloning.

Because of the popularity of some triploid genotypes since their generation, clonal propagation over centuries has created more or less large subgroups. Whether this genetic or epigenetic variation, or a combination of both, is still a matter of speculation. It is expected that the GBS technique will help clarify this situation.

Each subgroup has received the name of its best known member, which has become its representative cultivar (Table 3.1).

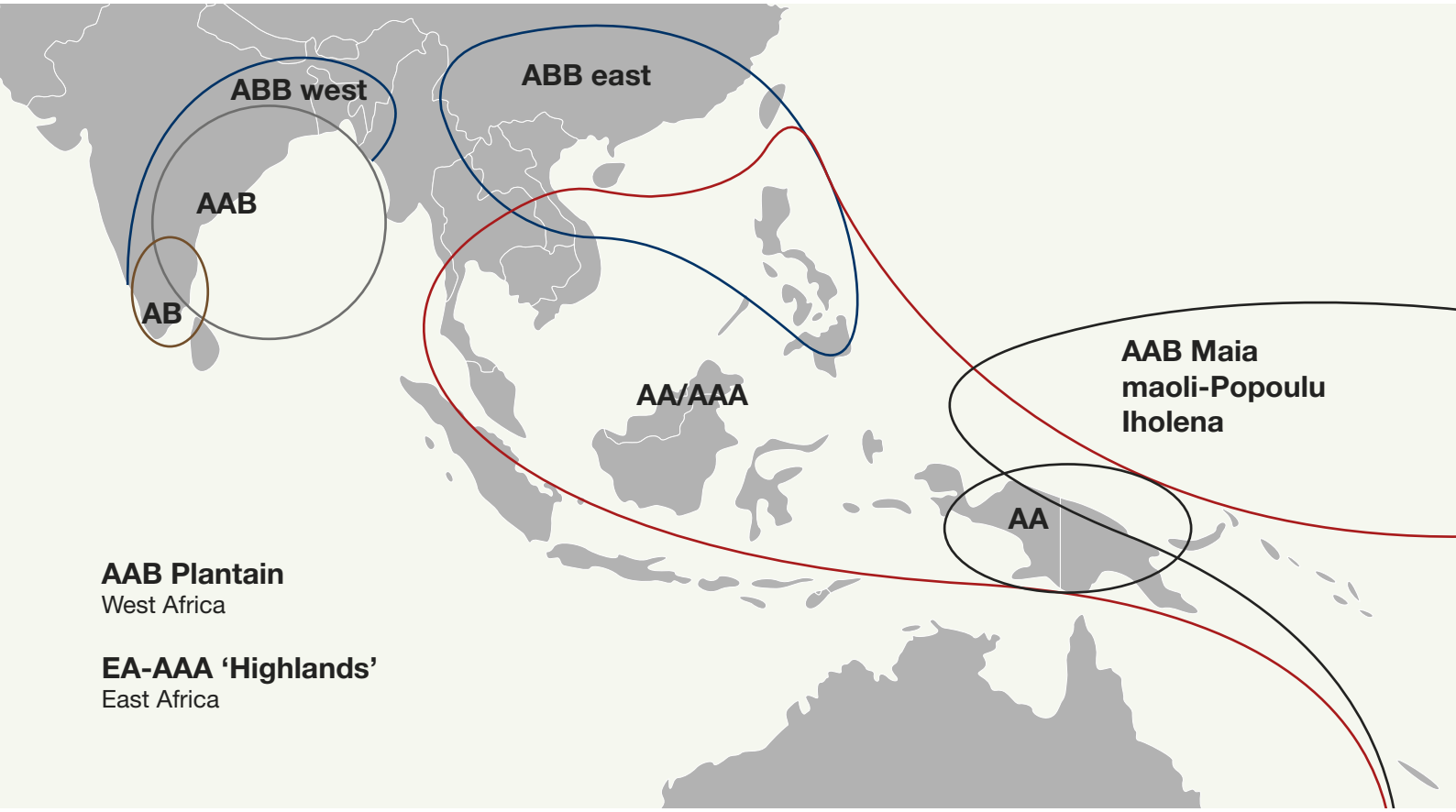
Table 3.1. *Triploids subgroups and their assumed centres of origin.*

	Genome group	Subgroup	Assumed Centre of Origin
1	AAA	Cavendish	Mainland S.E. Asia
2		Gros Michel	Mainland S.E. Asia
3		Red	S. E. Asia
4		Ibota	Island S.E. Asia
5		Rio	Mainland S. E. Asia
6		East African Highland AAA (beer cvs)	Great Lakes region
7		East African Highland AAA (cooking cvs)	Great Lakes region
8	AAB	Pome / Prata	India
9		Silk	India
10		Pisang Kelat	India
11		Mysore	India
12		Pisang Raja	Mainland S.E. Asia
13		Plantain- French/False Horn/ Horn	West and Central Africa
14		Maia Maoli/Popoulu	Polynesia, Melanesia and Micronesia
15		Iholena	Polynesia, Melanesia and Micronesia
16	ABB	Bluggoe	India
17		Monthan	India
18		Peyan	India
19		Ney Mannan	India
20		Pisang Awak	Mainland S.E. Asia
21		Pelipita	Philippines
22	AAA or BBB	Saba	Philippines

It is commonly accepted that triploids have been generated by spontaneous hybridisations of edible AA with various sources of the additional A or B-genome (other edible AA or wild AA, and *M. balbisiana*). Indeed, the female gamete production in edible AA displays many irregularities, including the entire restitution of the diploid genome during meiosis, as reported in the 1940s (Dodds and Simmonds 1946). When pollinated by A or B genome pollen, the thus fertilized egg cells would – rather exceptionally – simply contain the addition of AA+A versus AA+B and thus form AAA or AAB hybrids. The generation of ABB is still a matter of conjecture because the production of unreduced AB gametes may have been very rare as edible AB cultivars are so rare. Hence the suggestion that backcrosses played a more important role than previously assumed (De Langhe et al. 2010). The backcross hypothesis could also explain why some AAB or ABB cultivars show morphologies much closer to *M. acuminata* or *M. balbisiana* than the A/B ratio would dictate, opening the possibility of A/B allele exchanges 'underway'. Totally different direct triploidy formation is also likely in several cases. For example, dessert type ABB cultivars such as Peyan and Pisang Awak have a B cytoplasmic DNA strongly suggesting a BB x AB origin (a BAB constitution (Carreel et al, 2002).

The success of triploids, such as the AAA subgroup Cavendish, is due to their high vigour and fruit growth rate, as well as gametic sterility. For AAB or ABB, the *M. balbisiana* genome is long since known to add characters like tolerance to abiotic or even biotic stresses (Cheesman 1948). These favourable characteristics led to an extension far beyond the primary centre of diversity (Figure 3.1).

Figure 3.1. *Geographical distribution of where the edible AA and the main triploid banana cultivar groups/subgroups dominate. (Source: De Langhe et al. 2009).*



Since almost all triploids are sterile, their traditional presence and diversification in such distant regions is necessarily due to human interaction in the past. Elucidating the nature and period(s) of this interaction is the task of multidisciplinary research, of which the results could explain local preferences for one or other subgroup.

3.1.2 Constraints in triploids

Although banana triploids are relatively vigorous plants, the subgroups are more or less exposed to particular pests and/or diseases, depending on their genotype. This is certainly the case over recent times because of increased worldwide migration, where migrants act as carriers of contaminants.

The soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*) race 1 decimated the commercial AAA Gros Michel plantations in the Americas during the first half of the 20th century, while the AAA Cavendish subgroup appeared to be resistant in the same regions. However, the current Cavendish plantations are now seriously menaced by another – possibly more recently generated – *Foc* tropical race 4. The fungal leaf spot disease caused by *Mycosphaerella fijiensis* is seriously compromising the productivity of the AAB plantain subgroups. More devastating is the banana bunchy top virus (BBTV), which eventually can kill entire subgroups in some areas, such as AAB Prata in India, AAB plantains in Africa and the Pacific.

In many cases, the effect of the disease is amplified by a growing pressure from specific pests such as the nematode *Radopholus* and *Pratylenchus* species, or specific borer-weevils, which considerably weaken the defensive capacity of the host plant.

In the end, it can be assumed that any subgroup could be menaced in the future by bacteria, fungi, viruses, or other pests. The hardy ABB subgroups may show tolerance, in that the symptoms of the attack are less visible for a long time, but they eventually can succumb as well.

Climate change can lead to longer dry periods, to which AAA subgroups are particularly susceptible. Sustained dry periods have thus led farmers in East Africa to replace the Highland AAA cooking subgroup by hardier ABB subgroups in the most exposed areas, with the result that beer-making from these ABB bananas is progressing, because of their durable productivity.

Among other possible impacts of climate change, a predictable effect is an increase incidence of pests and diseases.

In summary, as an effective means to combat many of the above threats, the genetic improvement of bananas should be directed towards the sustainability of production, allowing the adoption of new cultivars with acceptable agronomic performance, pest and disease resistance and fruit quality required by the market.

SECTION 3.2 TRIPLOIDS - WHERE WE WANT TO GO

The improvement of the triploid cultivars calls for an exhaustive assessment of their taxonomy.

The total number of existing triploid cultivars in the traditional context is roughly estimated at around 1,000. Given that the cultivars are members of subgroups with different genotypes, genetic improvement of threatened cultivars is concentrated on one or a few ‘popular’ members of their subgroup. While the new hybrids do not display the diversity of the subgroup, they are readily accepted by the farmers for their resistance to pests and diseases. Besides, much of that subgroup diversity concerns various plant/fruit colours and fruit forms, characteristics that are of rather minor importance on the local market. The fruit form of the export bananas is the exception.

However, the taxonomic situation of numerous cultivars is far from clear. A standardized description of the representative cultivar per subgroup does not exist. This limitation has created the paradoxical situation that numerous cultivars have been individually described according to the standardized Descriptor List published by IPGRI-INIBAP and CIRAD in 1996 (see Chapter 5 - *Characterization*), but these could not be systematically linked to the subgroups. Too much still depends on the pragmatic insight of experienced taxonomists specialised in one or other particular subgroups and/or in its presumed wild and edible AA

ancestors. The result is that current breeding programmes are still relying on a few, rather incidentally 'known' sources, and do not harness the total diversity spectrum when selecting convenient material (see Chapter 12 - *Genetic Improvement*).

The strategy for achieving a completely satisfactory taxonomy of the triploids is developed in Section 3.3 below and further in Chapter 4 - *Taxonomy* and Chapter 5 - *Characterization*.

SECTION 3.3 TRIPLOIDS - HOW WE WILL GET THERE

Currently the most important tasks concerning triploids are the following:

1. Achieve the standardized description of all triploid subgroups.
2. Systematically classify the numerous cultivars per subgroup via their available descriptions. An important side result of this undertaking will be the correct classification of several 'floating' triploid cultivars, mostly collected in New Guinea and S.E. Asia, which have not yet been assigned to a subgroup.

The Taxonomic Reference Collection Project and its follow-up: a long-term programme

The highest priority is attached to the above tasks (1) and (2). To this end, a long-term programme will progressively cover the entire range of *Musa* germplasm, with the representative cultivars of the triploid subgroup in a first project, the "Taxonomic Reference Collection (TRC) Project" (see more details in Sections 4.3.2.1 - *Subgroup determination – the Taxonomic Reference Collection Project* and 5.3.1 - *The Taxonomic Reference Collection (TRC) Project*).

With this solid base, the programme will be extended to include the intra-subgroup diversity in a second project. In a third project, the standardization effort will embrace the edible diploids and wild ancestor subspecies/cultivars. It is expected that the programme should eventually also be able to classify ill-defined cultivars, subgroups and edible AA clusters.

PART B

IDENTITY

Part B covers *Musa* plant identity in two chapters. Chapter 4 focuses on *taxonomy* of both wild and edible bananas, including sections on explorations and collecting missions and cultivar identification down to the infra-subgroup level. Chapter 5 covers morphological and molecular *characterization*, down to the subgroup level, featuring different tools and their use, improvement of the descriptor lists, and using ethno-geographical information to guide cultivar identification.

CHAPTER 4. TAXONOMY

In whatever research or development enterprise throughout the world, any potential user searching for a suitable *Musa* cultivar needs complete documentation of its exact identity and if possible, its performance. The subject of performance and documentation is developed in Part D – Use (Chapter 9 - *Information Management* and Chapter 11 - *Evaluation of Germplasm*).

The identity of a cultivated or a wild taxon consists of its internationally adopted name (nomenclature), its description and its classification. Morphological description should be complemented with molecular-marker data, and both types of description are commonly called 'characterization'. The combination of *Musa* characterization and classification allows for the identification of any plant across the diverse *Musa* genus. The usual term "taxonomy" covers all these components as well as the implied research thereof. The term "systematics" is used to describe the search for phylogenetic relations within the *Musa* diversity.

This chapter identifies the many cases where characterization plays a key role in *Musa* taxonomy, while Chapter 5 reports the characterization methodologies.

SECTION 4.1 TAXONOMY - WHERE WE ARE NOW

Musa taxonomy has been a subject of research since the 19th century, at first overlapping with the significant period of botanical investigation of tropical flora and collections in botanic gardens, and later increasingly in agronomic research stations. By the mid-20th century, a fairly comprehensive knowledge base of both the wild and the cultivated diversity existed (Simmonds 1955 and 1962). However, it was admitted that the detailed diversity of wild and cultivated *Musa* germplasm had not sufficiently been explored, particularly for the subspecies and cultivars of *M. acuminata* and for the hundreds of cultivars grown in (sub-) tropical villages. In 1988, a state-of-the-art review of *Musa* taxonomy was presented during an international workshop at Los Baños, Philippines, leading to the identification of the major constraints facing *Musa* taxonomists and recommendations to overcome them (INIBAP 1990).

4.1.1 Taxonomy of wild bananas

The most important wild ancestor of edible bananas, *Musa acuminata*, has been extensively studied. While the species has been clearly defined, it displays an exceptional intra-specific variability, which causes classification problems. Since Simmonds (1956), the major variation has been classified as 'subspecies' with the adopted definition that they are "*morphologically distinct populations that occupy distinct geographical areas and breed mainly with themselves; that is, they are species in the genetic though not in the formal taxonomic sense*" (Simmonds 1962). They are not species in the classical sense since they have been proved to be inter-fertile, and since hybrids have been observed at the boundaries of their respective domains. Figure 1.3 in Chapter 1 (*Natural distribution of the* M. acuminata *subspecies and of the species* M. balbisiana (Perrier et al. 2011)), shows the geographical aspect of the subspecies concept.

However, the variation within each subspecies has hardly been searched for and systematically studied and is thus nearly absent in the *ex situ* collections. Yet, some of the incidentally *in situ* observed plants of several subspecies produced more impressive bunches than the currently used specimens in breeding programmes. The primary question of whether such stature is of genetic origin rather than due to favourable growth conditions has never been investigated.

Moreover, the lack of insight on intra-subspecific variation causes difficulties in the interpretation of observed surprises regarding the geographical boundaries of the subspecies. This can be illustrated with two examples:

a. The results of a series of seven reconnaissance missions across Thailand carried out by De Langhe, Volkaert and Watchanachaingcharung during the 1990s (INIBAP 2008), revealed that: (1) plants of the subspecies *truncata* were observed on lowland in the extreme south, while the habitat for this subspecies in Peninsular Malaysia is a typical highland one; (2) populations of an unrecorded *malaccensis* cultivar were found in valleys of the western mountains, far remote from Peninsular Malaysia where the subspecies dominates in lowlands; and (3) it appeared impossible to clearly distinguish the subspecies *burmannica* (western mountains) from *siamea* (north, northeast and east Thailand) because of overlapping infra-subspecific variations in bunch and male-bud shapes.

b. During a recent *Musa* exploration in Eastern Indonesia (Sutanto et al. 2015) plants resembling the subspecies *microcarpa* were observed in the Maluku province, with large fingers on bunches of up to 17 hands, a feature which has never been recorded in Borneo where the subspecies largely dominates. Part of the observed morphological variation of this 'microcarpa' seems to overlap with variation in the more eastern subspecies *banksii* (which dominates in the New Guinea region), to the point that one can presume a coherent set of closely related *M. acuminata* cultivars extending from Borneo to New Guinea.

It can be concluded that the infra-specific variation of *Musa acuminata*: (1) is far more complex than is understood from its subspecies classification, and (2) can provide more promising parent forms/cultivars for breeding than have been exploited until now.

Musa balbisiana, the wild co-ancestor of many edible banana subgroups, displays a rather restricted variation, and mainly in the generative parts. Nonetheless, some forms in South East Asia, such as cultivars Pacol and Butuhan in the Philippines (Valmayor et al. 2002) have apparently not been recorded in South Asia (India, Sri Lanka), a fact which points to subspecies existence. Efforts for assembling and studying the wild BBs in the Philippines have not delivered the desired clarification. Yet, these 'eastern' BBs may be at the origin of ABB subgroups that are distinct from the globally-spread Indian ABBs (Figure 1.4 - *Origins and migrations of the main triploid subgroups*). One also cannot yet exclude that genomes of these 'eastern' BBs may have contributed to the formation of the AAB plantains subgroup which spread in Africa and of the AAB 'Maoli-Popoulu' and 'Iholena' subgroups in the Pacific. In an exhaustive molecular genotyping using 22 SSR loci across 561 *Musa* accessions (Hippolyte et al. 2012), it was found that "*the balbisiana diversity provided by the interspecific cultivars seemed larger than the BB diploid diversity present in our BB samples …, suggesting an under-representation of the whole balbisiana diversity in collections or extinction of the BB parents of the current hybrids.*" Only coordinated molecular genotyping of all recorded and collected *balbisiana* forms could allow for the exact identification of the B- origins in the numerous AAB and ABB subgroups.

The taxonomy of the many other *Musa* species may seem of rather academic interest, but some aspects of it have quite a practical significance. A robust classification of wild *Musa* taxa was established in the mid-20th century by Cheesman (1948), Simmonds (1956) and Argent (1976) which has not been significantly modified since (see Figure 1.2 - *Distribution of the four sections of the genus Musa*). However, based on molecular findings, a simplification has been proposed in that Eumusa and Rhodochlamys would form one Section and Callimusa and Australimusa another (Wong et al. 2002; Christelová et al. 2011; Hřibová et al.

2011; Häkkinen, M. 2013). While the first 'new section' reflects an already common acceptance, the second new section concept may be debatable, mainly because of the unique seed form in Callimusa. In any case, the genetic distance of Callimusa-Australimusa vis-à-vis Eumusa-Rhodochlamys has convincingly been confirmed by molecular analyses mobilizing many species, especially members of the two distant sections, as is shown in Lin-Feng Li et al. (2010) and Janssens et al. (2016).

New classification uncertainties have emerged after several new specimens were recently found and described, principally in mainland South East Asia and in Indonesia. The taxonomic status of these new taxa is not clear, whether species, subspecies or botanical varieties. The proposed solution for better classification of wild germplasm is to revisit the section status, to complete the assessment of species/subspecies and to verify new wild accessions.

Indeed, this relative state of confusion hampers studies of the phylogenetic relations between the perhaps more than 50 *Musa* species and their subdivisions. Yet a clear understanding of the evolution of all these taxa could be of great help for genetic improvement when it comes to detecting useful traits and their alleles that are lacking or non-expressed in *M. acuminata* and *M. balbisiana*.

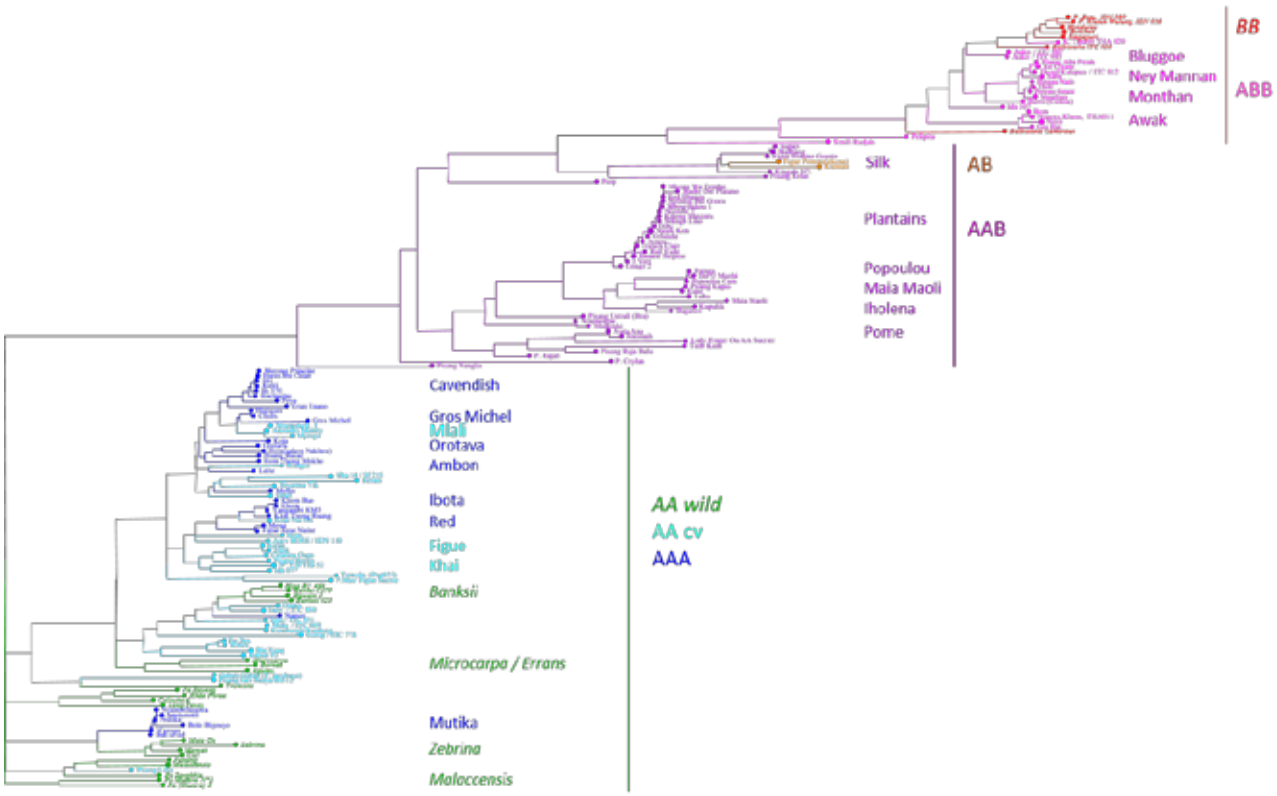
4.1.2 Taxonomy of edible bananas

Taxonomists estimate that there may be around 1,000 different *Musa* cultivars (INIBAP 2006). The names of these cultivars are not useful for classification; indeed, it can be stated that there exist as many distinct local names for a same cultivar as there are tribes or village communities (with their own language or dialect) that grow them. This largely unresolved synonymy problem means that field and *in vitro* banana collections are likely maintaining several replicates (synonyms) of many accessions. Sorting out these multiple synonymies can only be effective when the taxonomic situation of the corresponding cultivars has been definitively settled.

4.1.2.1. Taxonomy of the triploid bananas

For the meaning of the terms 'Group' and 'Subgroup', see Annex H - *Glossary* and Section 3.1.1 - *Triploid bananas as we know them*. Because of their widespread acceptance in the tropics over recent centuries, the most popular AAA, AAB, ABB cultivars had been fairly well classified and typified by their salient characteristics (Simmonds 1955). Since then, their respective morphologic variants have been classified as subgroups (see Table 3.1). An attempt at cultivar classification at subgroup level has been published by Stover, R.H. and Simmonds, N.W. in 1987 but is far from covering the entire diversity in subgroups, hence risking wrong identification. However, molecular genotyping has confirmed the genetic reality of the subgroup concept (see Figure 4.1). The afore-mentioned exhaustive SSR analysis by Hippolyte et al. (2012) has even added practical guidelines for correct attribution of a cultivar to some particular subgroups.

Figure 4.1. *Neighbour-Joining tree of 93 triploids with 836 DArT markers (Source: Risterucci et al. 2009).*



Uncertainties remain on the classification of a number of cultivars. For example, among the AAA, some cultivars are clearly different from the well-known subgroups Cavendish, Gros Michel, Red, Ibota (Yangambi Km5), and they seem to form one or more supplementary subgroups. It is expected that molecular genotyping, in combination with due plant description will eventually solve such uncertainties.

However, the molecular techniques that have been used so far (SSR, DArTs) have not been able to differentiate the cultivars within the subgroups. The subgroup ramifications in the derived cladograms (Figure 4.1) do not reveal morphological differences, and this also means that synonyms cannot be detected. As is suggested in Hippolyte et al. (2012, p13): "*… the low number and in most cases the absence of genetic differences between the accessions of a subgroup cannot explain the huge phenotypic diversity observed within these subgroups… Therefore other possibilities, such as epigenetic regulation, need to be explored.*"

Morphological taxonomy of the intra-subgroup diversity is therefore of the utmost importance. This is especially the case for the large and very ramified subgoups such as the AAA 'Cavendish', 'Gros Michel' and 'East-African Highland bananas', the AAB 'Plantain' and 'Maoli-Popoulu' and 'Iholena', the ABB 'Bluggoe', 'Monthan', 'Pisang Awak', and ABB/BBB 'Saba', which together represent the vast majority of cultivated bananas in the tropical and subtropical world. In each of these subgroups, the variants are of different nature. The AAA Cavendish types are connected to the dwarfism expression; the AAB 'Maoli-Popoulu' types deal with the fruit form; the types of AAA East-African Highland bananas are marked by several bunch and fruit traits

The most complex case is the AAB plantain subgroup, with probably more than 150 different cultivars. The subgroup consists of three widely different morphotypes: “French plantains” with a completely developed male inflorescence, “False Horn plantains” with a much reduced male inflorescence and much longer fruits, and “Horn plantains” with no male inflorescence at all and with the largest fruits of the entire *Musa* genus (see Figure 4.2).

Figure 4.2. *The three types of Plantains (Source: Adheka 2014).*

French Plantain

False Horn Plantain

Horn Plantain

While the French plantain type displays a wide variation in bunch structure, fruit forms, pseudostem and fruit colours, in all sorts of combinations, the variation exists partly in the two other types as well. But molecular genotyping (using SSR and DarT markers) cannot distinguish among these three major types, which suggests their all belonging to one subgroup.

The concept of ‘clone sets’, centred on the most representative/popular cultivar per type is handy for an initial intra-subgroup classification. Numerical morpho-taxonomy results on the AAB plantains and the AAA East-African Highland bananas display ‘clusters’ of cultivars that correspond fairly well with such clone sets (Karamura 1999, De Langhe et al. 2005, and Karamura et al. 2012). The intriguing question of whether clone sets have a different genetic background or a purely epigenetic one has not been elucidated by any current molecular studies. Indeed, they could be the derivatives of different siblings from one or more crosses between the same two parents of each subgroup, with slightly different allele sequence-combinations as a result, stemming from different meiosis configurations in the gamete formation. Different clone sets could also originate from a single sibling and in this case would have subsequently evolved in ‘different directions’ through somaclonal mutation and/or epigenetic variation.

Nevertheless, by definition, the members of a subgroup do share a large number of characteristics and descriptors. The latter being typical of the subgroup, the ideal identification of each cultivar should at the onset refer to the description of the subgroup to which it belongs, and then deal with the remaining descriptors for differentiating the cultivars, via clone-set description or directly (for small subgroups).

However, no description per subgroup is yet available. While the popular representative cultivars of many subgroups have been fairly well described for a long time, their variants are only documented in the form of the many individual complete descriptions of the corresponding subgroup cultivars, without any link to that

of the representative (in other words, as if each cultivar is a separate entity of the entire triploid population). Synonymy detection remains problematic or impossible on the base of such disorderly documentation.

4.1.2.2 Do BBB triploids exist?

A number of triploid cultivars in countries such as Thailand, Vietnam and the Philippines have a striking resemblance to the wild BB and have been proposed to form one or more BBB subgroups. The question whether they are actual BBB or ABB is still open. This is important because: (1) BBB existence would imply the seedless/parthenocarpy potential in B genome as well as in the A-genome, with interesting new prospects for banana improvement; and (2) these cultivars could be the product of complex back-crossings leading to "ABB" with very few remaining A chromosomes or alleles in the A-genome, the rest being replaced by B chromosomes or alleles. Unfortunately, an elaborate morphological description of the supposed BBB cultivars has not yet been officially reported, but a catalogue of the Saba cultivars found in the Philippines combining morphological descriptors and descriptive pictures is currently being compiled.

4.1.2.3 Taxonomy of the edible AA cultivars

Figure 2.1 captures the vast majority of the recorded edible AA cultivars, including possible synonyms. The clusters of close neighbours have provisionally received the name of one of their cultivars. The dessert AA cultivars in South East Asia (Khai, Figue, Buaya) are well known, but names are arbitrarily selected for the other clusters, which for the most part produce starchy bananas. The entire cluster series from Beram to Spiral is of Papua New Guinean origin, while Mlali is the cluster of AA cultivars found in East-Africa and nearby islands in the Indian Ocean.

In contrast with the triploids, these clusters cannot be considered as 'subgroups' because they may contain a mixture of somatic variants, synonyms and actually different genotypes. Hence the provisional name of "clusters". Efforts for a firmer classification of the edible AA are hampered by a combination of two causes: (1) their genetic background and (2) the poor progress made in a standardized morphological characterization of the cultivars. Because edible diploids have maintained a higher fertility than triploids, inter-cultivar crosses would have allowed for several further recombinations and hence a larger diversification through sexual recombination. While in triploids the diversification is rather restricted to somatic variation via century-long vegetative propagation.

However, the detected edible AA clusters have as yet not been morphologically described in a systematic way, so that a coherent classification is still lacking. The description is insufficient and/or heterogeneous all over the region where the AAs are grown. The description of the more than 100 AA accessions from Papua New Guinea is limited to the most striking features (Arnaud and Horry 1997). The many AA cultivars in Indonesia have been described for their essential characteristics, but the data were published in Malay only (Sutanto and Edison 2005). The Philippine AA cultivars have been described to the same degree by Valmayor et al. (2002). Many of them are grown in island and mainland South East Asia under various names. A very useful initial synonymy table has been published (Valmayor et al. 2000) but it can only be consolidated and expanded after all these cultivars are described and sufficiently illustrated by using a standardized system.

An effort has recently been launched by ProMusa in 2013 to address the synonyms issue in general. The banana cultivar checklist (http://www.promusa.org/Banana+cultivar+checklist) aims at compiling all known names, in any language, for a given cultivar. But extensive work still remains to be achieved in this area.

A progressive improvement of the description system will allow for systematic comparison of the morphotype and the genotype per AA cultivar and should eventually lead to the construction of an adapted but reliable classification, which would optimize the use of edible AA, whether directly or via genetic improvement.

4.1.2.4 Taxonomy of the AB cultivars

As already mentioned (Section 2.1 - *Edible Diploids – where we are*), these cultivars are very rare and their recorded presence is confined to South India (two subgroups at the most) and Southeast Asia-New Guinea (a couple of described cultivars at the most). This led to the assumption that their role in the formation of AAB and ABB triploids might have been minimal.

However, four edible diploids with mixed A- and B-genome characteristics have recently been found in Eastern Indonesia (Sutanto et al. 2014). SSR analysis positioned them in a same clade as the Indian AB cultivars. These AB cultivars, together with the Auko of Papua New Guinea and at least one presumed AB in the Philippines may thus have been instrumental in the generation of some hybrid AxB triploids in this region.

SECTION 4.2 TAXONOMY - WHERE WE WANT TO GO

4.2.1 Wild bananas

1. The first priority is the complete assessment of the diversity in *M. acuminata* and *M. balbisiana*, because of its direct and great potential influence on genetic improvement.
    a. For *M. acuminata*, the subspecies concept needs to be revisited. The primary question of whether intra-specific variation is of genetic origin rather than due to favourable growth conditions has never been investigated and, in the (molecular) genetically proved cases, botanical varieties should be clearly defined. The underexplored regions are being or will be systematically revisited: South East Indonesia, Myanmar, extreme Northeast India, as well as intriguing *M. acuminata* outliers in islands near East-Africa (Pemba, Madagascar) and Oceania (Samoa) (*see* Section 4.3 – *Taxonomy – How to Get There*). The thus completed documentation on wild *M. acuminata* will eventually solve the structure of its diversity and phylogeny.
    b. For *M. balbisiana*, a systematic collection and/or identification of specimens, especially in altitudinal Southeast Asia and the Philippines is still needed. A coordinated/standardized molecular marking of all recorded and collected *balbisiana* forms, in agreement with their morphological differentiation, is necessary for correct classification. This should lead to a reliable phylogeny of the many AAB and ABB subgroups and the use of proper *balbisiana* parents in breeding programmes.
2. The second priority is the solution of the 'Section problem', the first-level classification of all the *Musa* species (see Section 1.1 – *Wild Relatives and domestication – Where We Are*). Should the combined approach "morphology x molecular analysis x phylogeny" consolidate the term in some way, or should one search for an alternative? Several wild populations have recently been studied in South China for Eu*musa* and in Borneo for Callimusa (e.g. Häkkinen M. and C. Meekiong 2005; Häkkinen M. and Wang, H. 2007). They can be of great help in solving the question, provided their nomenclature and classification is internationally agreed.

4.2.2 Edible bananas

The evident priority is the correct morphological classification of any of the perhaps 1,000 existing cultivars. Indeed, the molecular investigation of cultivar genotypes can only produce reliable results when the identity of each involved cultivar is unequivocal and correctly named.

A. The triploids: The effort consists of four steps:
    i. Standardize the characterization of the representative cultivar of each subgroup, by both morphological and molecular means.
    ii. Standardize the morphological description of all the supposed member-cultivars of each subgroup.

iii. Per subgroup, to extract from the step 'b' above the descriptors that are common to all its cultivars, thus allowing the complete description of the subgroup.

iv. The remaining descriptors should differentiate any member-cultivar. Cultivars that apparently do not fit may belong to another subgroup or could be singletons.

B. The AA diploids: The priority is to sort out what part of the 'clusters' identified by molecular marker techniques could be actual subgroups, and then to decide on the nomenclature of the taxonomy for the entire group. The undertaking consists of following steps:

i. Standardized morphological description of all the members of each cluster found by molecular means. Verification of the many presumed synonyms by planting and comparing the concerned accessions in a same collection.

ii. When members of such cluster are morphologically different and therefore cultivars (somatic variants) in the same sense as those in triploid subgroups, they would constitute an actual subgroup and should enter the classification process as for triploids.

C. Other diploids: Since the ascertained and presumed AB cultivars have been collected from widely dispersed places (India, Indonesia, Papua New Guinea) an across *ex situ* collection survey (descriptions and photos) should produce a more coherent view on the group. This work should be complemented by analysing their diversity via molecular markers and comparing the results with the morphological descriptions.
The results of all these activities will enable the progressive identification of the duplicates and synonyms in field collections and at the ITC (see more in Part C – Management, Chapter 6 – *Musa collections around the world* and Chapter 7 – *The ITC Global Musa Collection*), In addition, it will allow the identification of the existing different AB and potential edible BB.

D. Verifying the possible existence of BBB triploids: The morphologically most convincing subgroup of the 'Saba' cultivars in the Philippines should be molecularly screened for any presence of specific A alleles or A contribution to the genome (genotyping, in situ hybridization, etc.).

E. Hierarchical identification of any cultivar: This is the ultimate goal of the above activities. The most logical and parsimonious cultivar identification should proceed along the hierarchical sequence of "Group to Subgroup to Cultivar", certainly for the triploids. A supplementary level may become necessary for the more complex edible AA (see Section 4.1.2.2 - *Taxonomy of the edible AA cultivars*) once their taxonomy has been settled.

i. Identifying the Group (AA, AB, AAA, AAB, ABB; and BB, BBB if their existence is confirmed): Locating any cultivar in one of the groups on morphological grounds has its pitfalls. The ploidy level should be ascertained or adjusted by flow cytometry, as the morphological signal of erect leaves in diploids is not always reliable, and some diploids can even look like the vigorous triploids under favourable growth conditions. Furthermore, molecular markers can reveal the presence of an unexpected A or B genome (or at least one or several such alleles and even chromosomes) in triploids, thus pointing to a more complex generation than the expected genome-proportions from morphological observation. For example, a few triploids with 34 chromosomes, due to unbalanced chromosome restitution during meiosis (23 chromosomes instead of 22 given by one parent) may have arisen from possible backcrosses. Therefore, a more exact chromosome counting by the MGC can be necessary for assessing such cases. The classical group identification is based on the scoring system of Simmonds and Shepherd, which used 15 morphological descriptors, each in states 1 to 5, with 1 = pure *acuminata* and 5 = pure *balbisiana* . But their goal was to demonstrate the existence of the different genome-balances in AA/AAA, AB, AAB, ABB, and not for the location of any cultivar in one of the groups. When it comes to locating any cultivar in a group, one can immediately perceive the risk of the intermediary scores, since the choice between states 2, 3 and

4 (respectively rather like *acuminata*, just between *acuminata* and *balbisiana*, rather like *balbisiana*) relies too much on the subjective judgement of the observer. Such morphological identification process needs more than 15 descriptors, and current efforts are trying to find the reliable group-differentiating ones.

ii. Identifying the Subgroup: Once the subgroups are identified and characterized for triploids and edible diploids, a Determination Key should be constructed for facilitating the identification of the subgroup to which an observed cultivar belongs (for example, develop/identify sets of descriptors that could be used to assign accessions to subgroups) .

iii. Identifying the Cultivar: Once cultivars are differentiated, as stated above in Step A (iv), an intra-subgroup determination key can be constructed for exact cultivar identification in each triploid subgroup. For large subgroups, the key could have to use an intermediary 'clone set' concept. The remaining descriptors should differentiate any member-cultivar. Cultivars that apparently do not fit may belong to another subgroup or could be singletons.

SECTION 4.3 TAXONOMY - HOW WE WILL GET THERE

4.3.1 Explorations and collecting missions

The following collecting/exploration missions have either been achieved or are planned or recommended.

1. Triangle Exploration in Southeast Indonesia
   A team of *Musa* taxonomy specialists searched for unrecorded wild and edible bananas in the triangle Sulawesi-Maluku-Lesser Sunda. It confirmed the existence of an *acuminata* subspecies in Sulawesi, which had been previously named 'cultivar *tomentosa*' (Nasution 1991) and of which several characteristics are also found in ssp. *banksii*. It found several wild *acuminata* populations in Maluku that slightly resembled either the subspecies *microcarpa* or *banksii*, but no 'new' subspecies. The results thus suggest a distinct and very complex diversity with the probable extremes in Borneo (typical *microcarpa*) to the west, in the Philippines (*errans*) to the North and in New Guinea (*banksii*) to the East. The complex seems morphologically strikingly different from all other *acuminata* subspecies in western Indonesia and up to Thailand (except perhaps for the ssp. *truncata* in highland Peninsular Malaysia, and the ssp. *sumatrana*, both of which slightly resemble *microcarpa*). All recorded specimens are presently growing in the collection at the Indonesian Tropical Fruit Research Institute (ITPRI). DNA obtained from leaf samples was investigated. The exploration mission also collected a number of unrecorded edible diploids and triploids. Ploidy assessment by flow cytometry proved that some assumed triploid plants (including 'plantain-like' cultivars) are in fact diploid. The findings are reported in a contribution to the 2014 ISHS ProMusa symposium (Sutanto et al. 2015). However, the authors insist on the need for subsequent local explorations which should find several other unreported taxa in that region.

2. Myanmar
   The exploration in Myanmar is planned and is pending agreement from the national authorities. While no great surprises are expected concerning edible bananas, the configuration of several Eumusa species within this country, including little-known *acuminata*-like taxa (e.g. *M. flaviflora*) makes it a key region for elucidating the Eumusa diversity.

3. Extreme North East India
   The extreme north east of India could not be regularly explored during previous expeditions (Uma et al, 2005) because of political unrest. Its interest is similar to that of Myanmar, but it is also said to host a number of presumed edible BB and BBB. The exploration calls for a coordinated planning with the Indian authorities and specialists.

4. Indonesian New Guinea
   This province of Indonesia has merely been subject to incidental observations and collections, mostly along the coastal areas. Because of the intermediate position between the well-known diversity in PNG and the recently recorded diversity in Eastern Indonesia (above item 1), a rather modest exploration project would solve the following questions:
   a. Are there unique cultivars of AA subspecies *banksii*?
   b. Is there introgression of *banksii* with the *acuminata* subspecies complex of Eastern Indonesia and/or Philippines and its genetic significance?
   c. Confirmation of the western limit of *M.schizocarpa*? According to the taxonomist Jeff Daniells (pers. comm.), a large population exists between Jayapura and the PNG border but no trace was found around southern Timika. The species could already be absent in the province of West Papua (hence with no ad hoc edible *acuminata x schizocarpa* hybrids as result).
   d. Are there region specific edible AA and various triploids?
5. East Africa
   Seed-bearing *M. acuminata* has been reported in Madagascar and Tanzania (island of Pemba). There are questions linked to the origin of the seeded plants, if they were introduced by human, and how they may link to the cultivated diploid 'Mlali' accessions. It is also worth noting that in northwest Malawi and the area between northern Mozambique and southern Tanzania there are some edible diploids of *Musa acuminata* which need to be collected and characterized. Another objective is to discover the nearest wild diploids to the East African Highland bananas for the crop improvement programme in East Africa.
6. Near Oceania: Papua New Guinea – Solomon Islands
   The region of Papua New Guinea hosts 8 to 9 wild *Musa* species unique to the New Guinea and under-represented in genebanks. In addition, unique Fe'i cultivars remain uncollected both in the mainland and east islands including the Solomon Islands. Finally, not all the diversity of cultivated bananas has been collected in the mainland and some islands of the Bismark archipelago and of the Solomon Islands need to be explored.

4.3.2 Cultivar identification

4.3.2.1 Subgroup determination – the taxonomic reference collection (trc) project

As explained in Section 4.2.2 – *Edible bananas*, key to achieving the determination of subgroups is the standardized characterization of the representative cultivar of each subgroup (by both morphological and molecular means). To this end, 34 accessions, representing all popular edible subgroups, including both diploids and triploids, plus wild diploid taxa (see Table 4.1), were selected from the ITC to constitute the Taxonomic Reference Collection (TRC), and were distributed to a number of collaborating institutes with field collections (Table 4.2).

Table 4.1. *Current Composition of the Taxonomic Reference Collection – 34 accessions.*

	ITC code	Accession name	Species or Genome group	Subspecies or Subgroup
1	ITC0249	Calcutta 4	acuminata	burmannicoides
2	ITC0766	Paliama	acuminata	banksii
3	ITC1177	Zebrina	acuminata	zebrina
4	ITC0247	Honduras	balbisiana	(type 1)
5	ITC1120	Tani	balbisiana	
6	ITC0312	Pisang Jari Buaya	AA	Pisang Jari Buaya
7	ITC0653	Pisang Mas	AA	Sucrier
8	ITC1121	Pisang Lilin	AA	Pisang Lilin
9	ITC1187	Tomolo	AA	(Cooking AA from PNG)
10	ITC0245	Safet Velchi	AB	Ney Poovan
11	ITC0081	Igitsiri (Intuntu)	AAA	Mutika/Lujugira (beer)
12	ITC0084	Mbwazirume	AAA	TE
13	ITC0277	Leite	AAA	Rio
14	ITC0575	Red Dacca	AAA	Red
15	ITC0654	Petite Naine	AAA	Cavendish
16	ITC0662	Khai Thong Ruang	AAA	Ibota
17	ITC1122	Gros Michel	AAA	Gros Michel
18	ITC1287	Pisang Berangan	AAA	Philippine Lacatan/Sgr. 555
19	ITC0109	Obino l'ewaï	AAB	Plantain (French)
19b	ITC0121	Ihitisim	AAB	Plantain (Horn)
20	ITC0450	Pisang Palembang	AAB	Pisang Kelat
21	ITC0519	Obubit Ntanga green mutant	AAB	Plantain (French)
22	ITC0587	Pisang Rajah	AAB	Pisang Raja
23	ITC0649	Foconah	AAB	Pome / Prata
24	ITC0769	Figue Pomme Géante	AAB	Silk
25	ITC0825	Uzakan	AAB	Iholena
26	ITC0335	Popoulou	AAB	Maia Maoli/Popoulou
26b	ITC1169	Mai'a popo'ulu moa	AAB	Maia Maoli/Popoulu
27	ITC1325	Orishele	AAB	Plantain (False Horn)
28	ITC1441	Pisang Ceylan	AAB	Mysore
29	ITC0123	Simili Radjah	ABB	Peyan
30	ITC0361	Blue Java	AAB	Ney Mannan
31	ITC0472	Pelipita	AAB	Pelipita
32	ITC0659	Namwa Khom	AAB	Pisang Awak
33	ITC0767	Dole	AAB	Bluggoe
34	ITC1483	Monthan	AAB	Monthan

Table 4.2. *The 12 collaborating institutes with field collections participating in the TRC Project*

Country	Institute
Brazil	EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária
Burundi	IRAZ - Institut de recherches agronomiques et zootechniques (IRAZ) de la CEPGL
Cameroun	CARBAP - Centre Africain de Recherche sur Bananiers et Plantains
Costa Rica	CORBANA - Corporación bananera Nacional
India	NRCB - National Research Centre for Banana
Indonesia	ITFRI - Indonesian Tropical Fruit Research Institute
Nigeria	IITA - International Institute of Tropical Agriculture
Philippines	BPI - Bureau of Plant Industry
Tahiti French Polynesia	SDR-MAP - Service du développement rural
Uganda	NARO – National Agricultural Research Organisation
USA, Puerto Rico	USDA-ARS – United States Department of Agriculture, Tropical Agriculture Research Station, Puerto Rico
Vietnam	FAVRI - Fruit and Vegetable Research Institute

The selection of this set of 34 accessions was based on the following criteria:

- Representativeness of morpho-taxonomic variation within the subgroup.
- Available for distribution from the ITC collection (virus-indexed negative).
- Declared true-to-type during field verification, cytogenetic and molecular studies.

Standardized morphological description and photography are carried out on the TRC according to the rules explained in Chapter 5 - *Characterization*. The set of four wild taxa is a taxonomic framework for controlling some difficult descriptions.

The results are compared with those of molecular marker techniques at the MGC for substantiation/ reconciliation. Analysis on datasets obtained from the morphological characterization of this unique set of accessions in the different collections of the project is expected to begin in 2016. It is expected to allow i) improving the quality and understanding of the descriptors by multi-testing the whole set of descriptors, ii) identifying those of the descriptors that are stable or not across environments and iii) producing and releasing a set of accessions multi-described and multi-documented to be used world-wide as a reference for taxonomic and training purposes.

The TRC project is thus the backbone operation for the classification of the entire edible banana spectrum (except the taxonomically very distant Fe'i), and a large part of Chapter 5 – *Characterization* deals with this. The adopted strategy is to run the project in combination with the cultivar numerous descriptions (details in the next section).

4.3.2.2 Infra-subgroup differentiation: Cultivar identification

Conforming to the goals stated in Section 4.2.2 - *Edible bananas*, this rather long-term activity relies on the collaboration in the Regions where the different subgroups dominate.

The AAB Plantains in West and Central Africa.

- In DR Congo, a large collaborative effort is ongoing between the Universities of Kisangani, Butembo, Bioversity, and the Katolieke Universiteit Leuven (K.U. Leuven) via several projects. The effort aims at covering the entire Congo-Basin, including the middle altitudes in Kivu province. At present about 100 plantain cultivars have been collected and described, most of them having many synonyms. Despite

the existence of the widely different classical 'clone sets' French, False Horn, and Horn, a general description of the subgroup has been constructed (Adheka 2014).

- CARBAP in Cameroon is developing a network of national plantain collections in West Africa with support from the European Union. The programme has the same objectives of further collecting, standardized description and synonymy clarification of the many cultivars.
- The planned coordination of the two above undertakings will allow for the total coverage of the entire plantain diversity in Africa.
- On the molecular side, since neither SSR nor DArT are able to differentiate infra-subgroup cultivars, it is hoped that the next generation techniques such as GBS and RADseq produce more convenient data (see Section 5.3.3).

The AAA-EA East-African Highland AAA bananas (also named "EAHB)

This is a collective name for at least two subgroups, Mutika-Lujugira and Ilalyi. The latter subgroup produces rather 'cucumber-like' fruits with an unusual rounded apex, and some of its members are quite reminiscent of AAA cultivars recorded in PNG. The very large Mutika-Lujugira subgroup of about 60 cultivars has been extensively studied (Karamura 1999; Karamura and Pickersgill 1999; Pickersgill and Karamura 1999) and has since been subdivided in five 'clone sets': Mfuka, Nkabululu, Nakitembe, Musakala, beer bananas. Molecular markers (SSR, DArT) tend to put all these cultivars in one cluster. The concept of the fifth set of beer bananas has now been abandoned (Kitavi et al. 2016). Indeed these cultivars do belong to one or more of the other clone sets.

AAB subgroups 'Maoli/Popoulu' and 'Iholena' in New Guinea and the Pacific

These subgroups have until recently been underexplored, but current widespread and systematic cultivar exploration/collection/description is progressively clarifying their constitution. The existence of two clone sets in the first subgroup (resp. Maoli and Popoulu) has been confirmed and also that of 'intermediary' cultivars, thus showing a complex pattern with some cultivars as candidate 'relicts' of a common ancestor. These could deliver the basic descriptors of the subgroup determination. The Iholena subgroup seems elusive in that it contains many "Iholena-like" cultivars for which molecular markers produce more than one cluster. Determination of this subgroup thus requires a (more) coordinated programme and support.

ABB Bluggoe-Monthan-Bontha complex in South Asia.

More than 200 cultivar names in India belong to this morphological complex, in which intermediary forms make the subgroup determination quite difficult. While ABB-focused molecular studies have as yet not been reported, general genotyping through SSR and DArTs do not clearly differentiate the complex. The better performing SNP markers could be of considerable help in searching for a solution.

ABB/BBB/BB in mainland and island Southeast Asia.

While it will not be difficult to construct the determination of the popular and ubiquitous 'ABB Pisang Awak' subgroup, all other cultivars of this large B-genome dominated cultivar assembly – including the supposed existence of edible BB and of BBB cultivars - are in an uncertain taxonomical situation. This is the remaining large gap in the assessment of edible banana diversity. Yet its potential is undeniable given that these cultivars are likely to contain unexploited B-genomes or alleles. A coordinated programme at regional scale with appropriate support should address this serious deficiency.

Edible AA in Island Southeast Asia and the New Guinea region.

As explained in Section 4.1.2.3, the interpretation of clusters produced by molecular marker techniques is seriously hampered by the lack of a satisfactory morphological classification of the perhaps 200

edible AA. Again, only a regionally coordinated programme can solve this problem, along the steps as suggested in Section 4.2.2.

The table below summarizes the future objectives activities described in this chapter.

Table 4.3. *Summary of proposed objectives and actions related to* Musa *taxonomy.*

	Objectives	Proposed actions
1	Fully assess the diversity of *M. acuminata* and *M. balbisiana*	• Set up collecting missions to: Myanmar, Extreme North India, Indonesia New Guinea, East Africa, Near Oceania • Study the diversity of wild genepools with molecular markers
2	Refine the taxonomy of triploid cultivars	• Identify subgroup discriminative descriptors through the multi-environment characterization of the TRC • Identify subgroup specific descriptors through the extensive characterization of targeted subgroups
3	Revise the taxonomy of diploid cultivars	• Characterize the accessions composing the molecular clusters and assess if they compose subgroups • If so, agree on subgroups names
4	Explore AB diversity	• Perform a survey of the AB in *ex-situ* collections (with descriptors and photos) • Molecular analysis of these AB

CHAPTER 5. CHARACTERIZATION

SECTION 5.1 CHARACTERIZATION - WHERE WE ARE NOW

Characterization is the basic tool for the classification of any cultivar. Both characterization and classification constitute its identity (see introduction of Chapter 4 - *Taxonomy*).

5.1.1 Morphological characterization

Morphological characterization is the precise qualitative description of the plant when growing in optimal conditions whereby its characteristics are maximally expressed. Qualitative description is confined to those descriptors that are minimally or not at all influenced by geographical variation in climate and soils, i.e. highly heritable. The different possible states of each descriptor are morphological markers. Moreover, in the case of cultivated bananas, which are clonally propagated, the markers never change over time, except for somatic variants. In addition, these may include a limited number of additional [quantitative] traits thought desirable by a consensus of users of the crop.

Descriptors for Banana (*Musa* spp.) was published in 1996 by Bioversity (then IPGRI and INIBAP) jointly with CIRAD, in consultation with several *Musa* taxonomists, to allow for a standardized terminology to be used for description during explorations *in situ*) or *ex situ* as the accessions is entered in field collections (IPGRI-INIBAP, CIRAD, 1996).

The list contains descriptors for the standard recording of passportdata,collecting,management,andenvironmentand site descriptors. These are followed by 121 characterization descriptors with their different possible states. For example, with the descriptor 6.3.20 “colour of midrib dorsal surface” (of the third, fully unfolded leaf counting from the top of the plant), the f ollowing states are distinguished: yellow (1), light green (2), green (3), pink-purple (4), red-purple (5), purple to blue (6), other (7), with explanation by the observer). The list also contains 35 evaluation descriptors (susceptible to environmental differences) and 11 descriptors for markers and cytological characteristics.

This descriptor system has been adopted by the curators of field collections. But since many descriptors deal with colours or forms with sometimes delicate gradation (e.g. ‘light green’ versus ‘green’) there is a risk of different interpretations by different observers, that effectively can lead to ‘artificial’ differences between described cultivars or accessions. Some definitions of descriptor-states may also have to be made more explicit or more adapted to infra-subgroup diversity, and would benefit from specific illustrations. This is still seriously hampering the correct documentation. The measures to overcome the problem are explained in Section 5.2.2.

5.1.2 Molecular characterization

Molecular characterization proceeds by finding those DNA sequences of which the variation (polymorphism) reveals differences between cultivars. However, because detected variations can occur within non-coding regions and because the genomic role of the *Musa* sequences found with common marker techniques is as yet not known for the most part, it would be erroneous to consider these markers as reflecting the morphological ones. Molecular characterization correlates to some extent with taxonomy based on morphology, but is also a complementary tool for complete characterization.

The molecular marker techniques currently used for *Musa* description are the SSR, the DArT, and the SNP, which are explained in the glossary.

A GBS (genotype-by-sequencing) project for the sequencing of very large number of plantain and plantain-like accessions has been launched and may enable in the future progress in the conciliation of morphological and molecular characterization data at the intra-subgroup level.

Another technique, the RADseq (Restriction-site Associated DNA sequencing), allowing the genotyping of more SNPs sites has been recently used for a widest diversity analysis with a focus on the diploids.

Meanwhile, SSR markers are most useful because of their reliability and transferability between laboratories. Currently a set of 22 SSR markers are widely used for banana molecular characterization (e.g. Hippolyte et al. 2012; Christoleva et al. 2011; Irish et al. 2014) and allow distinguishing subgroups and subspecies.

DArT markers are handy for genome-wide analyses (see Figure 4.1 - *Neighbour-Joining tree of 93 triploids with 836 DArT markers*, as an example). Despite the dominant nature of DArTs, they can be used to compare different genomes at a large number of loci in a single assay. DArT markers are able to spot accessions which are not grouping with what was expected. In many cases DArT analysis allowed to complement a classification (e.g. the subgroup of a poorly-identified accession can be identified).

Both SSR and DArT have largely confirmed the morphological classification down to the subgroup level, and proved to be very helpful in solving remaining classification problems. But they are generally not adequate for infra-subgroup differentiation of cultivars and are thus of no help for completing their characterization.

SNP marker techniques are becoming cheaper but are cumbersome in analysis. However, the analysis of a large number of SNPs may be of considerable help in elucidating the intra-subgroup differentiation problem when the nature of the differentiation is genetic.

Both morphological and molecular characterization methodologies are progressively moving towards a single harmonious system adapted to the special nature of edible banana diversity. Indeed, it should be re-emphasized that the clonal nature of the estimated 1,000 banana cultivars prevents the taxonomist from simply adopting the methods and organisation developed for seed-propagated crops where the cultivated races are selected genetically-moving populations.

SECTION 5.2 CHARACTERIZATION - WHERE WE WANT TO GO

Characterization as a toolbox for classification of taxa should be markedly improved and developed in order to assess the entire *Musa* diversity.

For the morphological characterization, an iterative process is proposed that allows for simultaneous progress in (1) characterization of the subgroups and of their cultivars and (2) optimal performance of the descriptor lists, including their adaptation to the cultivar differentiation within the different subgroups.

For the molecular characterization, stress is put on (1) optimal marker performances for cultivar differentiation within subgroups and (2) complete conciliation between the found cultivar-sample differences and the corresponding morphotypes.

The expected result will be the reliable characterization of *Musa* taxa at all diversity levels.

5.2.1 Determination of the Subgroups

The Taxonomic Reference Collection Project (see Sections 4.3.2.1 and 5.3.1) should produce the firm base for the morphological characterization of all edible bananas, i.e. the standardized description of the representative cultivar per classically known subgroup. The simultaneous description of the cultivars in each subgroup will allow the determination of its variation-limits, hence a clear and coherent inter-subgroup differentiation. The synthesis of all these results should be the Hierarchical classification of any cultivar, thus simplifying its identification via determination keys, such as Musa.ID, which is described in Chapter 9 – *Information Management*.

5.2.2 Improvement of the Descriptor Lists

The use of the Descriptors booklet (IPGRI-INIBAP, CIRAD 1996) in several collections has produced in many cases contradictory descriptions for a same cultivar distributed by the same source such as the ITC. This constraint considerably hampers the documentation of the accessions and data exchange through the *Musa* Germplasm Information System (MGIS) (see Chapter 9 – *Information Management*) and hence their proper use. The system can only be improved if the causes of these differences are identified and addressed. Photos enable users to revisit each description. A major part of the solution is constructing well-defined and user-friendly descriptor lists, as well as the exchange of description results among curators to help clarify differences in interpretation.

5.2.3 Optimal use of SSR and DArT results

Classifications of cultivars via morphological descriptions should agree with the findings via molecular techniques for the same cultivars. The results of SSR and DArT techniques should be widely diffused among all interested collections for due interpretation by the curators who perform the morphological descriptions. Their comments should be subject of a regular dialogue with the molecular specialists, in order to reach complete agreement on any cultivar characterization.

However, it should be recognized that the available clonal germplasm, which has now been repetitively analysed, represents only a portion of the genepool. While the ITC - the standard source for these techniques - contains a sufficient diversity in clonal accessions, it still lacks some of the wild sources, especially on the level of taxonomic varieties and of populations in *M. acuminata* and *M. balbisiana*, which should be anchor points for comparative genotype interpretation.

Hence the absolute need for collecting these wild sources, as explained in Chapter 1.

5.2.4 Highly performing molecular techniques for cultivar differentiation within subgroups

An ongoing project based on SNPs, the “Genotype-By-Sequencing (GBS)” project (see also Section 5.3), focuses on the large AAB Plantain subgroup. Project data are being analysed and will hopefully reveal ability to detect polymorphism that could be linked to morphological traits. If not, lack of sequence-polymorphism at the intra-subgroup level would point to epigenetic origins of variation, and in that case invite post-transcriptional research.

The Genomics Thematic Group (GTG) of MusaNet has also proposed to investigate *Musa* germplasm genetic diversity through resequencing. Such data may potentially reveal chromosomal structural variations, copy number variation and enable identification of the dispensable genome/pangenome.

5.2.5 Ethno-geographical information is a guide for cultivar identification

Local people use names in their own language for plant taxa with which they are familiar with, and certainly for their traditional banana cultivars. These names are primarily based on distinct features so that the local nomenclature can reveal discriminative descriptors. The names can also refer to the way people use the fruit or other parts of the plant and this aspect is of interest to ethno-botanists. Comparing the local taxon with taxa from the region can frequently point to its initial origin and hence help in the reconstruction of its history. That knowledge can have interesting genetic implications such as from where and how the cultivar originated, thus underlining the practical value of multidisciplinary research.

SECTION 5.3 CHARACTERIZATION - HOW WE WILL GET THERE

5.3.1 The Taxonomic Reference Collection (TRC) Project

This item addresses the above points in Section 5.2.1 *Determination of the Subgroups* and Section 5.2.2 *Improvement of the Descriptor Lists*.

Recognizing that a standardized subgroup determination is the backbone for the entire edible banana classification, the *Musa* taxonomy community embarked several years ago on the TRC Project, which is focused on the description of the representative cultivar for each of 29 classical subgroups (i.e. 34 accessions in total including 5 accompanying 'control' accessions, see Table 4.1).

5.3.1.1 History

The (*Musa*) Taxonomy Advisory Group (TAG), at its 2006 meeting in Cameroon, recommended that a Taxonomic Reference Collection of the cultivars representing as much as possible the entire spectrum of edible banana diversity be established in field collections in the different regions, as a tool to help resolve some taxonomic issues and improve the usability of the morphological characterization descriptors.

Objectives of the TRC project:

1. To test the robustness of the standardized descriptors on the selected accessions across the participating field collections.
2. To identify environmental factors that may influence the plant morphology.
3. To promote the full and satisfactory characterized accessions of the TRC as reference and standards for all other cultivars in every participating field collection.

These objectives will contribute towards the goal of improving the usability of the descriptors. The preparation of this enterprise on a worldwide scale took quite some time. All the corresponding accessions were to be delivered by the ITC in order to guarantee the strict homogeneity of the material (thus preventing many equivocal situations). Meanwhile, curators were duly informed on the purpose of the operation and the incentive for them and their institutes for responsible collaboration.

Eventually, a TRC list was adopted at the subsequent TAG meeting in 2008 in India, and an initial number of curators confirmed their participation. Thereafter, the TRC project entered its operational phase, coordinated by Bioversity. Over the following years, other curators joined the TRC, and ITC started the multiplication *in vitro* and the preparation of hundreds of plantlets. These were progressively sent in several batches per participating collection, and grown in the fields via nurseries.

The TRC project is presently in the phase of analysis of the first results (1st Cycle).

5.3.1.2 TRC Project Methodology

Technical guidelines for the multi-location characterization of the 34 TRC accessions listed in Table 4.1 were distributed to ensure that the description is done uniformly at all sites (see www.musanet.org). Field collections in 12 institutes participate in the TRC project and they are listed in Table 4.2.

The guidelines make recommendations on the agronomic practices, the planting layout, density and distance and data to be recorded for the first and second cycles including environmental data from the weather station nearest the trial site.

Rooted plantlets (4 per accession) were sent by the ITC in several batches and grown in nurseries before planting on the field. The set of each accession is observed for at least two vegetative cycles, at appropriate growth phases. The descriptions from the first cycle along with the appropriate pictures are compiled by Bioversity.

The description for the second cycle is the definitive one, should no longer be exposed to misunderstandings, and should produce the final characterization data for the accession. Once all the data are compiled by Bioversity, the appropriate statistical analysis will be undertaken to identify which of the descriptors are robust across the different environments of the project. From these results, a consensus characterization of the TRC should also arise. All the characterization and documentation data produced within this project will be available online through the MGIS. Such results will further allow a collective deliberation on the adapted description of all possible members of the respective subgroups.

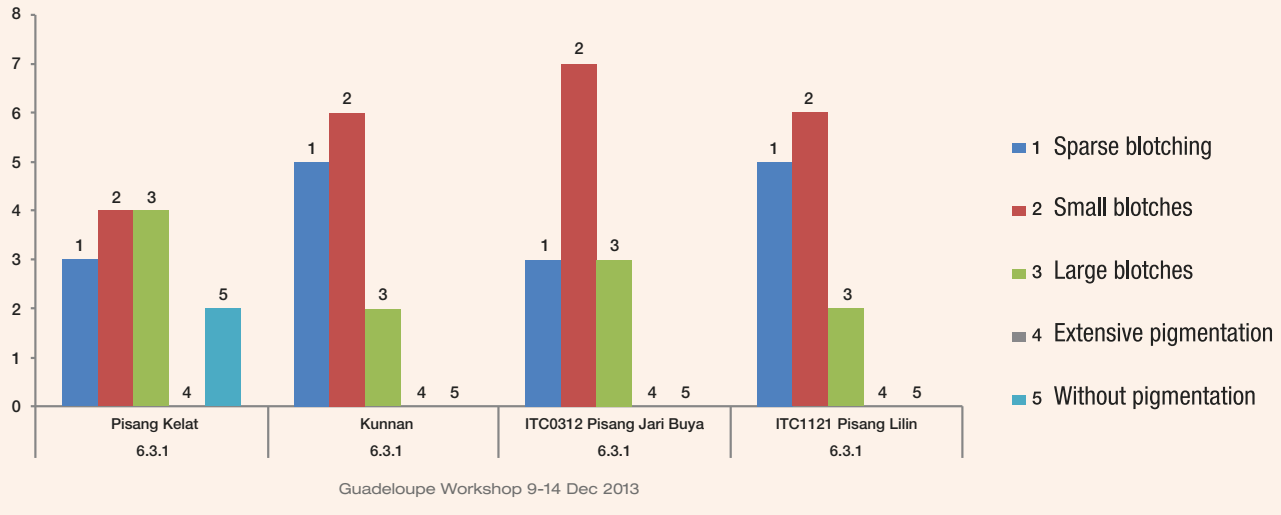
5.3.1.3 Current situation

To date, the TRC project has passed the 1st cycle in most of the partner collections and the minimum descriptors are now being compiled.

In December 2013 MusaNet organised a workshop to address the most urgent needs of *Musa* collection curators vis à vis the management of germplasm and its associated information. It included ensuring the correct description of the most difficult characters of the TRC. The workshop took place in the field collection of the CIRAD in Guadeloupe, French West Indies, where more than 300 different accessions are growing under optimal conditions.

The TRC project field exercise was focused on the above mentioned reason (1), “interpretation”, i.e. the choice between the descriptor states as explained and illustrated in the Minimum Descriptor List of descriptor states, and this for 20 difficult descriptors. Two days were spent to the strictly individual description of 4 accessions by 13 observers, and to an extensive discussion of the results. The exercise undeniably revealed major interpretation contradictions for several descriptor states. A striking example was the descriptor 6.3.1, Blotches at petiole base (Figure 5.1)

Figure 5.1. *Results from descriptor 6.3.1, Blotches at petiole base for 4 accessions, characterised by 13 observers.*



The demonstration proved that descriptor interpretation is the dominant cause of the contradictions, which would occur even in the ideal situation of uniform management across the collections.

The results of the Guadeloupe workshop led to a first updating/improvement of the Minimum List of Descriptors for *Musa*. Knowledge sharing sessions were also held on field management, data management and documentation. A prototype hand-held tablet was introduced to facilitate data collection in the field, and feedback was collected toward its development.

A follow up workshop of the TRC partners was held in December 2014 workshop at Trichy, India, at the National Research Centre for Banana (NRCB), where a wide range of *Musa* diversity is maintained. One of the primary outputs of the workshop was an agreement on the revised Minimum List of Descriptors for *Musa*, i.e. that they are interpreted and recorded in the same way by all curators. Recommendations were made on the next steps of the TRC project and on regional workshops to fine-tune the description of *Musa* subgroups.

5.3.1.4 Beyond the TRC Project: Descriptor Lists for infra-subgroup differentiation

In accordance with Section 4.3.2.2 - *Infra-subgroup differentiation: Cultivar identification*, special descriptor lists per subgroup are being drafted for the description of the subgroup cultivars, to be tested and eventually finalized via regional workshops. They combine relevant parts of the Minimal List of Descriptors for *Musa* with a variable range of additional descriptors pending the specific subgroup diversities. The latter are not only extracted from the complete descriptor list (INIBAP-IPGRI, CIRAD 1996) but consist also of descriptors (or additional descriptor states) that are rather unique to one or other subgroup. Meanwhile, the Minimum List of Descriptors for *Musa* underwent a second updating on the basis of the Trichy workshop feedback.

The Minimum List of Descriptors for Plantains was piloted during the May 2015 Regional MusaNet-CARBAP Workshop for West and Central Africa, at CARBAP in Njombe, Cameroon. This workshop built on the experience from the two previous MusaNet workshops, but with a regional scope. In attendance were 12 invited curators of national *Musa* collections across West and Central Africa and key experts.

In addition to the development of a new plantain specific descriptor list, the hand-held tablets and software were tested during the field characterization component of the workshop. The Minimum List of Descriptors for Plantains is available on www.musanet.org and the delivery of the final version of the tablet software is expected by mid-2016.

For more details, see the technical reports on the 2013 Guadeloupe, 2014 India and 2015 Cameroon MusaNet workshops at www.musanet.org.

In parallel to these workshops, Bioversity developed a tool to help characterize banana plants in the field (MusaTab, see Section 9.3.1). In addition, an updated tablet version of Musa.ID (formally MUSAID), a programme developed by CIRAD, will be released soon (see Section 9.3.1). Musa.ID aims to help identify cultivars through comparison with datasets of standard descriptors collected in selected field collections. In this context, the morphological characterization of the TRC in 12 different environments will certainly help increase the power of this tool.

5.3.2. Optimal use of SSR and DArT results.

5.3.2.1. The Musa Genotyping Centre – MGC

The *Musa* Genotyping Centre (MGC) was established in 2006 at the Laboratory of Molecular Cytogenetics and Cytometry of the Institute of Experimental Botany (IEB) in Olomouc, Czech Republic. The MGC was founded following discussions during the TAG 2006 meeting in Cameroon. MGC serves the community by genotyping *Musa* accessions and supports research activities of the Global *Musa* Genomic Consortium (GMGC), which recently became the Genomics Thematic Group in MusaNet. The methods applied at MGC are chromosome counting, karyotype analysis using tools of molecular cytogenetics, estimation of

ploidy and nuclear genome size by flow cytometry, genotyping using a set of 19 microsatellite markers (SSR; Christelová et al. 2011) and sequence analysis of internal transcribed spacers (ITS). The molecular characterization of *Musa* accessions using SSR genotyping approach provides reliable classification at subgroup level. ITS sequence analysis is used to confirm the results, if needed, and to verify genomic constitution in putative hybrids. The major goal of MGC is to characterize all accessions held at ITC and a Bioversity project is planned to further support this activity.

However, the genotyping service is provided to any researcher of the *Musa* research community wishing to have their material genotyped (the service is provided on a cost recovery basis; see http://olomouc.ueb.cas.cz/musa-genotyping-centre for more info). As the same SSR genotyping platform (Christelová et al. 2011) is used for all samples, this approach enables direct and precise comparisons of various accessions. Moreover, the precision of genotyping continues to increase with increasing number of accessions genotyped, as the representation of individual subgroups in the dataset is enlarged and strengthened. Compared to genotyping using high-throughput next-generation sequencing-based methods, the strength of SSR genotyping lies in its capability of systematic and long-term sample processing. New accessions can be analyzed and added to the already existing database of SSR profiles, while the optimized and not demanding methodology assures comparability of results gathered at different time points, and at reasonable cost.

5.3.2.2. Combined in situ *Characterization, a test case*

The Triangle exploration in Southeast Indonesia (see Section 4.3.1) has combined morphological characterization of the *in situ* observed taxa with systematic leaf sampling for ploidy assessment and SSR analysis at the MGC. While the SSR findings consolidated the tentative classification on morphological base for many wild specimens, several supposed edible triploids appeared to be diploid or the reverse, thus correcting their classification. By this procedure, the fully-fledged observations of the corresponding introductions in the *ex situ* collections are presently integrated into a rational framework, rather than being applied at random.

The combined *in situ* method is essential for wild taxa that are not thriving *ex situ* because they are accustomed to particular habitats (high altitudes, monsoon climate, and specific soils) which are not reproducible in the usual field collections. The most practical alternative is then the observation in National Parks where most of these wild taxa can be found, so that a simple GPS can locate them for any later observation.

5.3.3. Highly performing molecular techniques for cultivar differentiation within subgroups.

5.3.3.1. High-Throughput Genotyping techniques

Two high-throughput genotyping techniques are currently in use for studying *Musa* diversity: Genotyping-By-Sequencing (GBS) and Restriction-site Associated DNA sequencing (RADseq).

In addition to a set of accessions representing the *Musa* diversity, the GBS technique has been applied to a set of selected Plantains in the framework of a project involving Bioversity, CIRAD and the J. Craig Venture Institute (JCVI), and funded by USAID. The aim was to produce unique DNA fingerprints for each of the accessions and to better understand the processes that are involved in the diversification of this subgroup. The preliminary analysis of the thousands of SNP markers produced showed that molecularly differentiating the different Plantains is not straightforward, even when using the new technologies. Nevertheless, a full exploitation of such data's potential will require new tools, new methods and new approaches adapted to the problems linked to clonal diversification. These methods are currently under development.

RADseq applied to banana produced more SNP markers, allowing for a better coverage of the genome, but the differentiation within subgroups has not been thoroughly investigated yet. Bioversity International is producing such data for hundreds of banana accessions. The data should be analyzed in 2016.

5.3.3.2. Whole Genome sequencing

Through the African Plant Breeding Academy launched in December 2013, the African Orphan Crops Consortium (AOCC) will genetically sequence, assemble and annotate the genomes of 100 traditional African food crops, including banana. Among the 200 accessions of bananas that will be sequenced, 50 should be Plantains and related AAB cultivars. This huge effort in terms of production of data aims at guiding and speeding up the development of more robust production with higher nutritional content. However, the availability of the full genomic sequences of different cultivars of Plantains will also constitute a high-value resource to i) understand the diversification within subgroups and ii) develop specific, accurate markers to discriminate clones within subgroups.

In 2016, members of the GTG of MusaNet published an improved reference genome sequence for *M. acuminata* DH Pahang (D'Hont et al. 2012). Priorities in terms of genome sequencing include the development of reference sequences for additional species and subspecies, with a longer term objective of characterizing the pan and core *Musa* genome, together with nomenclature that enables genes to be distinguished according to (sub)species and accession origin.

5.3.4. Ethno-geographical information on cultivars

For any observed cultivar/accession, the passport data should not only include the village and GPS where it was first found, but also (1) the ethnic group and its language, (2) the carefully spelled local name and its significance, provided by the informer/interpreter. Linguists specialized in the local languages should be consulted for verifying these data and comments, which should enable the reconstruction of the history of the cultivar (e.g. loanwords indicate introduction from a nearby or even more remote areas). The comparative analysis of the assembled data for a subgroup in each area can be of considerable help in reconstructing its history in the region and beyond, thus providing hints on the genetic background.

The table below summarizes the proposed future activities discussed in this section.

Table 5.1. *Summary of proposed objectives and actions related to* Musa *characterization.*

	Objectives	Proposed actions
1	Assess which of the descriptors are robust across environments	• Multi-location characterization of the TRC • Statistical analysis of the results obtained
2	Identify subgroup specific descriptors	• Organize regional workshops dedicated to specific subgroup e.g. East Africa for EAHB
3	Ease the identification of cultivars – wild types	• Update Musa.ID
4	Optimal use of past work with SSR	• Pursue the molecular characterization of *Musa* diversity with SSR to enrich existing databases and reach a molecular picture of the whole *Musa* diversity
5	Molecularly differentiate cultivars within subgroups	• Test new techniques available • Investigate other approaches (e.g. epigenetic)

PART C

MANAGEMENT

Part C covers the management of *Musa* germplasm in three chapters. **Chapter 6** covers *Musa collections around the world* including field, *in vitro*, seed and cryopreservation. **Chapter 7** focuses on the *ITC global Musa Collection*. **Chapter 8** covers the areas of *in situ and on-farm conservation* of *Musa diversity*.

CHAPTER 6. *MUSA* COLLECTIONS AROUND THE WORLD

For a global system for the conservation and use of *Musa* genetic diversity to be effective, the entire genepool must be safe-guarded in perpetuity in *ex situ* collections, complemented by *in situ* and on-farm conservation. The wide range of correctly identified diversity should be accessible and available for use through the promotion of safe-movement of germplasm to any *bona fide* user.

Musa cultivars are usually seedless and options for their long-term *ex situ* conservation are constrained by the vegetative nature of the plant's reproductive system. So the *ex situ* conservation consist of mainly 3 types: (1) *in vivo* as full sized plants in field collections, (2) *in vitro*, as tissue culture on agar, in controlled environment/laboratory conditions and (3) in cryopreservation, at -196°C in liquid nitrogen. However, wild species produce seeds and their *ex situ* conservation through storing seeds is being studied.

The majority of the *Musa* germplasm is maintained *in vivo*, i.e. in the many field collections around the world in the centre of origin and in the main production areas and secondary diversity regions. The field collections are important for taxonomic characterization, evaluation, training and breeding purposes. The institutes maintaining field collections are therefore an essential part of a global system for the conservation and use of *Musa* diversity as they form the network of key partnerships. Field collections however are highly vulnerable to pests, diseases and natural disasters and need regular replanting (regeneration). They also need to be safely-duplicated, preferably in a different country/region.

Musa ex situ collections have been established since the first ones in Trinidad in the1920s, at INERA (ex INEAC), Congo DRC in 1933, and in Cote d'Ivoire in the 1940s. A major one was initiated by CIRAD in Guadeloupe in the 1950s. Then followed a gradual evolution of collections being created with the majority of them (37 out of 56) being established from 1990 to recently (2013). See Table D.3 in Annex D.

If *ex situ* conservation is to completely cover the entire existing *Musa* diversity, including all explored and collected germplasm as well as the germplasm yet to be discovered, there is a need to correctly identify and eventually fill the possible gaps across all collections. Adequate gap identification requires that accessions be duly characterized and documented, preferably at the site of their origin, before the material is introduced into collections. Not only is the initial data vital for further studies, but without proper documentation, collections run the risk of maintaining duplicates of what is already conserved elsewhere. Correct characterization entails more than just description. Researchers must frequently compare the observed and analysed taxa with several other taxa for final assessment. Consequently, the *ex situ* collections need to contain representative taxa across the range of diversity. This work has been carried out through the *Musa* Taxonomic Reference Collection (TRC) Project – for more information see Chapter 5 - *Characterization*.

The management of *Musa* germplasm *ex situ* collections consists of the following activities:

1. Conservation of acquired accessions (medium and long-term conservation, including safety duplication) – field, *in vitro*, cryopreservation.
2. Collecting and acquisition of new materials

3. Characterization for identity and distinguishing accessions
4. Preliminary evaluation for traits of potential interest for improvement
5. Germplasm health management
6. Genetic integrity management
7. Information management (including documentation and dissemination of information)
8. Germplasm distribution (including safe movement)

This chapter summarises the current status of *ex situ* collections, i.e. what is managed and how, what are the priorities for improvement, and how the strategy expects to meet these priorities. Its major focus is the activity of conservation of acquired accessions, while the other related activities are examined in the light of the following chapters for implementation at the *ex situ* collection level:

- Collecting, acquisition of new materials and gap filling - Chapters 1-3
- Characterization for identity and distinguishing accessions – Chapter 4-5
- Information management of *Musa* genetic resources – Chapter 9
- Distribution and safe exchange of germplasm – Chapter 10
- Evaluation for traits of potential interest for improvement – Chapter 11

SECTION 6.1 *MUSA COLLECTIONS AROUND THE WORLD* - WHERE WE ARE NOW

The information on the current status of the *Musa* collections worldwide presented below is drawn from feedback of collection curators through a global survey carried out from September 2012 (*see* Annex D - *MusaNet Global Survey of* Ex Situ *Collections – 2012-2015*). It also includes feedback obtained during a number of MusaNet consultations. Over 60 collections were contacted with the support of the Regional *Musa* Research Networks and MusaNet coordination to provide updated information on the following:

- Information on the Institute and Curator of Collection
- Content of the *Musa* Collection
- Germplasm Management
- Documentation and Information
- Germplasm Exchange and Dissemination
- Long-term Security of the Collection

By October 2015, 56 collections had responded to the survey (see Annex D - *List of Musa Collections that participated in the 2012-2015 Global Survey*).

The results are provided here and commented per activity as proposed in the above Introduction.

6.1.1 The Surveyed Collections

The locations of the surveyed collections are shown on the map in Figure 6.1 below.

Figure 6.1. Musa *ex situ collections around the world.*

Table D.2 in Annex D lists the institutes managing *Musa* genetic diversity *ex situ* and the total number of accessions (including field, *in vitro*, greenhouse and cryopreservation collections).

Most of the collections have been established over the past 30 years (see the year of establishment of the surveyed *ex situ* collections in Table D.3 in Annex D) with the earliest collection being INERA in Congo DRC, which was started in 1933. It is interesting to note that apart from the first collection at INERA, there is a gradual and continuous establishment of *ex situ* collections from the late 1960s to recently, with the following collections currently present in the 4 regions (based on the survey):

- Eastern Africa: 10 collections
- Western Africa: 7 collections
- Asia and the Pacific: 27 collections
- Latin America and the Caribbean: 9 collections
- Global collections: 2 collections (ITC-Belgium and IITA-Nigeria)

Of the 56 collections, 19 were established in the 1990s, probably corresponding to the push of countries to safeguard their own national resources stimulated by the Convention on Biological Diversity (CBD) adopted in 1992. And a further 18 were established since 2000, with the most recent being IRAF in Gabon, established in 2013.

6.1.2. Mandate and Priorities of Ex Situ Collections

The survey collected information the responsibilities of the institutes in maintaining *Musa* collections such as: ownership of the collection, government mandate, research and funding.

The large majority of the institutes (92%) stated that they own the materials in their *Musa* collection. And four collections stated that they do not own the germplasm, including the ITC, SPC, NBPGR and CORPOICA. The ownership of the germplasm is a complex issue in some cases and it can be argued that no single institute owns the germplasm that is maintained the public domain collections but is the legal guardian. This is particularly the case with the ITC collection, which belongs to the global community, and may be the case for the SPC, where the material is maintained on behalf of several countries in the region.

Most of the collections (77%) are officially mandated by their national government for the conservation of *Musa* and 93% to conduct research on *Musa*.

Of those with a mandate for conservation, most collections are for conservation at the national level. The following collections indicated also having also a mandate at the regional level:

Table 6.1. *Collections with regional-conservation mandates.*

	Country	Institute
1	Burundi	IRAZ
2	Cameroon	CARBAP
3	China	TSFR Lab
4	Cook islands	Ministry of Agriculature (MoA)
5	Côte d'Ivoire	CNRA
6	Fiji	SPC
7	French Polynesia SDR	Pacific Response Fund Committee
8	(PRFC)	Pisang Lilin
9	Nigeria	IITA
10	Papua New Guinea	NARI
11	Philippines	BPI
12	Puerto Rico	USDA
13	Sri Lanka	Horticultural Crop Research and Development Institute (HORDI)
14	Tanzania	ARI-Maruku
15	USA	Waimea

A few collections have indicated having an international mandate which may be interpreted as the materials being available for international distribution. The collections in the CGIAR maintained at Bioversity (ITC) and IITA are under the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA).

The main priorities indicated by the survey respondents for the collections are:

- genetic resources conservation - 88%
- support to breeding programmes - 49%
- characterization and genetic studies - 43%
- dissemination and distribution - 38%

Most of the collections (89%) are managed by publically funded institutes (government institute, university, etc.) and 83% of these are parties to the ITPGRFA. This would mean that in theory, if a collection is publically funded in a contracting party country, the material should be available to any *bona fide* user, although this is not currently the case. The constraints in the current system for the safe exchange of germplasm are discussed in Chapter 10 – *Distribution and Safe Exchange of Germplasm*.

6.1.3. Content of the Ex Situ Collections

According to the survey, the 56 responding institutes conserve over 9,051 accessions of *Musa* germplasm in field, 4,507 in *in vitro* collections, 898 accessions in greenhouses and 926 accessions in cryopreservation.

The majority of the materials in the collections are described as cultivars (71%) and a smaller proportion of wild taxa (13%) and breeding lines (9%) (see Table 6.2). More details for each institute are available in Table D.4 in Annex D.

Table 6.2. *Number of accessions of different types of germplasm in the field and* in vitro *collections.*

Collection type	Field Collections		*In vitro* / tissue culture Collections		TOTAL	
Cultivars	75%	5,783	64%	2,492	71%	8275
Wild taxa	12%	945	15%	599	13%	1544
Breeding lines	9%	660	9%	357	9%	1017
Others	4%	328	11%	442	7%	770
Total	**100%**	**7,716**	**100%**	**3,890**	**100%**	**11,606**

The survey collected information on the species and sub-species for the wild taxa conserved and 30 institutes provided lists of accessions. But in the absence of a complete list of accessions and details associated with each accession, it is not possible to draw any conclusion as to the identity of the materials conserved, nor to the amount of duplication that may exist within and among collections.

The survey also collected information on what makes the collection important or unique (from the collection curators' perspectives). The most frequent answer was related to *Musa* wild species and other more specific examples were: plantains, East Africa Highland bananas, indigenous cultivars, and accessions resistant to biotic and abiotic stresses, accessions from local or regional collecting missions that are found nowhere else and collections that represent a wide range of diversity within particular groups (e.g. sections or clone sets). Table D.9 in Annex D provides specific descriptions of germplasm from 41 collections.

More than 35 field collections each keep more than 100 living accessions, which is an expensive activity. *In situ* conservation of the cultivars has therefore been advanced as a partial alternative. Indeed, safeguarding the cultural heritage in traditional communities (from erosion caused by rapid economic development) should include the conservation of the banana cultivars in the villages, a form of *in situ* conservation. But farmers tend to reduce their cultivar stock in favour of (alien) more hardy or more productive cultivars, with the exception perhaps of the most remote populations. Furthermore, management differs by cultivar, with some cultivars surviving and thriving with minimal inputs, while others do not (referred to in Solomon Islands as 'long-term' and 'short-term' bananas). Therefore, the monitoring of cultivars grown on farms and in home gardens would be useful. However such an activity may be costly and difficult. This is further discussed in Chapter 8 - In situ and On-farm Conservation.

6.1.3.1 Conservation of wild species

Some wild germplasm accessions disappear from *ex situ* collections because field collections do not always provide the suitable ecology. This is frequently the case for species of the Australimusa and even

Callimusa sections and for those Eumusa species restricted to mountainous climates. Furthermore, wild species are sometimes better at seed propagation than vegetative propagation. This may be partly related to pests and diseases and access to a wider selection of genes, rather than one or two collected genotypes within the population.

The idea of wild sub-collections in appropriate environments is interesting but difficult to implement. The best alternative is *in situ* conservation of wild taxa, with geo-referenced passport data. This calls for a coordinated organization whereby the collectors and other parties (e.g. National Parks) play a crucial role (see Chapter 8 - *In situ and On-farm Conservation*).

6.1.3.2 Acquisitions, elimination and loss in the Collections

The survey suggests that an estimated 7,224 accessions have been acquired since 2002 and that 854 accessions have been removed from collections as duplicates or synonyms. But the information on the origin of the acquisition is not reliable enough to draw conclusions on the proportion of accessions coming from other countries or regions. It appears that the smaller collections have mainly acquired their materials from local farmers and in some cases from the country's wild areas (see Table 6.3). The larger collections have a greater proportion of materials coming from their breeding programme and introduced (i.e. not native to the country).

Information from 52 collections on 8,545 accessions shows the following:

Table 6.3. *Origin of the materials in the collections.*

Origin	Percentage
Introduced (not native)	48%
Coming from local farmers	32%
Collected from the country's wild areas	13%
Coming from the national breeding program	5%
Origin unknown	2%
TOTAL	**100%**

The issue on acquisition illustrated above is directly linked to the question of facilitating access to the material for other collections in the region, or for ensuring a duplicate is safely conserved at the global collection at ITC and available for use by all *bona fide* users. Accessing materials that have been introduced into the country should not be controversial and mainly be a question of having the mandate, and the technical and financial support to send out healthy materials upon request, since this material was introduced in the first place. Issues are more complicated when accessing materials from local farmers and/or from the wild. This is further discussed on Chapter 10 -*Distribution and Safe Exchange of Germplasm*.

The survey also enquired about accessions lost during the past 10 years or removed due to the change in the institutes' priorities but the data is not sufficiently standardised to be analysed conclusively. Information provided by several respondents highlighted some of the reasons for the loss or removal of accessions: i) because the material was introduced from another collection and is still available from that original collection and particularly the ITC; ii) they were known duplicates or synonyms, iii) they are common cultivars, or iv) the germplasm is available locally. Other reasons relate to damage such as pest and disease infected materials, particularly viruses, nematodes and weevils; floods and cyclones; drought, and physical damage to the genebank due to wars. Some losses were due to failure in the growth of the accession in the field or *in vitro*, acclimatization problems or lack of irrigation and low input levels. In some cases, the quarantine restrictions required the eradication of banana streak virus (BSV)-affected accessions or those related to completed projects researching topics such as disease resistance and evaluation.

6.1.4. Field Collection Management

Field genebanks provide easy access to plant genetic resources, for characterization, evaluation or utilization, while the same material conserved *in vitro* or in cryo must be regenerated and grown before it can be evaluated. Materials grown in the field are also important for conserving vegetatively propagated genotypes that commonly produce variants (genetic variation), since these can be more easily identified in the field than *in vitro*.

Out of the 56 collections surveyed, 52 are field collections conserving a total of 9,051 accessions (see Table D.2 in Annex D).

The Global *Musa* survey aimed to assess the current practices of the *ex situ* collections. It collected information on the size and planting spacing of the field collections and specifically on the following:

- Number of plants per accession
- Space between each plant of the same accession (*in metres*)
- Total size of field collection (*in hectares*)
- Space between 2 different accessions (*in metres*)

The total number of hectares of 47 collections who provided data is estimated at 68 hectares, with the average collections being of 1.5 hectares.

Out of 50 field collections, 28 institutes (56%) mentioned having an operation manual containing standard procedures and protocols for the management activities of the collections.

Data from 33 collections maintaining more than 100 accessions showed the following practices illustrated by Figures 6.2, 6.3 and 6.4 below.

Figure 6.2. *Number of plants per accession – data from 29 collections*

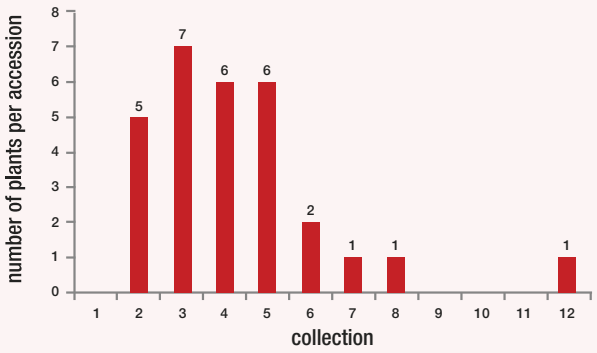


Figure 6.3. *Space between each plant of the same accession (in metres) – data from 32 collections*

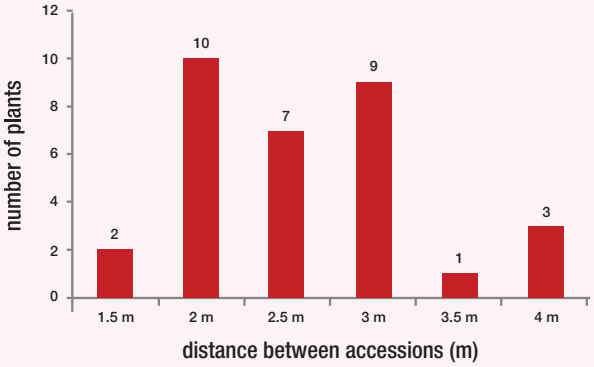
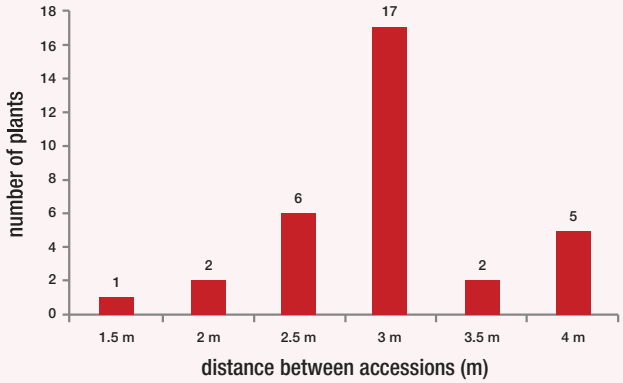


Figure 6.4. *Spacing frequency between 2 different accessions (in metres) – data from 33 collections*



The survey collected information on plant maintenance using agrochemicals, de-suckering, manual weed eradication, cover-cropping, mulching, fertilizers, compost and irrigation. The frequency of these management practices is detailed in Table 6.4 below, based on data from 49 collections.

Table 6.4. *Field management practices – data from 49 collections*

Field management practices	Regularly	Occasionally	Rarely	Never
Herbicide use	30%	34%	19%	17%
Pesticide use	15%	30%	34%	21%
Fungicide use	17%	21%	30%	32%
Weed eradication (manual)	69%	18%	8%	4%
Cover crop (renewed)	13%	21%	23%	43%
Mulching (other than banana leaves)	27%	24%	31%	18%
Fertilizer use	66%	23%	4%	6%
Compost use	30%	40%	19%	11%
Irrigation	50%	17%	15%	17%
Desuckering	64%	23%	9%	4%

Most of the field collections are replanted over periods of more than 2 years with 34% of collections transplanting less than 10% of their collection each year. Percentages of responses are in Table 6.5 below.

Table 6.5. *Frequency of replanting the field collections – data from Global* Musa *survey.*

Method	Every year	Every 2 years	More than every 2 years
Transplanting less than 10% of the collection	34%	13%	53%
Transplanting between than 10-50% of the collection	7%	36%	57%
Transplanting more than 50% of the collection	3%	10%	88%

According to the survey the most damaging biotic factors affecting the field collections are the following: Fusarium wilt, banana bract mosaic virus (BBrMV), banana bunchy top virus (BBTV), banana streak virus (BSV), cucumber mosaic virus (CMV), bacterial wilt, black leaf streak (BLS), other Mycosphaerella leaf spots, nematodes and weevils.

Abiotic factors such as drought, floods, extreme temperatures, high winds and poor soil conditions are mentioned as having a major effect in many cases. The details of collections experiencing major and minor effects of biotic and abiotic conditions are found in Table D.6 in Annex D.

The impact of pests and diseases affecting the safe distribution of materials in the collections is further discussed in Chapter 10 - *Distribution and Safe Exchange of Germplasm*.

Best management practices for maintaining a field collection varies across the type of *Musa* germplasm, environments and factors such as pests and diseases present. Efficient management also depends on the expertise. Therefore capacity building is important, particularly the sharing of knowledge and expertise with new staff and documenting the recommended practices for a specific collection.

In 2008, CARBAP developed a set of regeneration guidelines (found at http://cropgenebank.sgrp.cgiar.org/) and

it was suggested to include recommendations on additional practices such as de-suckering, management of grasses, cover crops and naked soils, and of diseases such as Black and Yellow Sigatoka.

6.1.5 In vitro Collection Management

In vitro collections are used mainly for the safety duplication of the field collections and for rapid multiplication and safe movement of disease-free planting material. It is also referred to as slow-growth conservation and requires regular sub-culturing. *In vitro* conservation in controlled growth conditions avoids infestation of the germplasm by pests and diseases, as well as climate shocks, which may affect field collections. Planting material for distribution can be obtained faster than from field plants and due to aseptic growth conditions, the health status, once determined, can be guaranteed. One drawback of *in vitro* conservation is that the material might be subject to somaclonal variation through spontaneous genetic mutation. Therefore, rejuvenation and verification of the trueness-to-type of the conserved germplasm has to be performed periodically. Furthermore, the problem of *in vitro* conservation of plantains and the presence of the banana streak virus (BSV) integrated into the B-genome may require the need for alternative approaches about their conservation. Recommendations for BSV-affected germplasm and distribution are discussed in Chapter 10 - *Distribution and Safe Exchange of Germplasm*.

The principles and guidelines for banana *in vitro* conservation are provided in Benson et al. 2011.

Out of the 56 collections from the Global *Musa* Survey, 32 institutes maintain an *in vitro* collection varying from a few accessions to the largest collection at ITC of 1,479 accessions. A total estimated 4,507 accessions are conserved *in vitro*. Table 6.6 below lists the collection with more than 100 accessions conserved *in vitro*.

Table 6.6. *Collection with more than 100 accessions conserved in vitro.*

	Country	Institutes Acronym	*In vitro collection – No of accessions*
1.	Global	ITC	1479
2.	Australia	DAFF-Mar	417
3.	India	NBPGR	415
4.	Brazil	EMBRAPA	250
5.	China	TBRI	220
6.	China	IFTR-GAAS	215
7.	Fiji	SPC	185
8.	Global	IITA-Nigeria	180
9.	Malaysia	MARDI	180
10.	Colombia	CORPOICA	164
11.	Burundi	IRAZ	155
12.	Puerto Rico	USDA	150
13.	Cuba	INIVIT	137
14.	China	TSFR Lab	110

The survey collected information on the existing *in vitro* collections, specifically on the following:

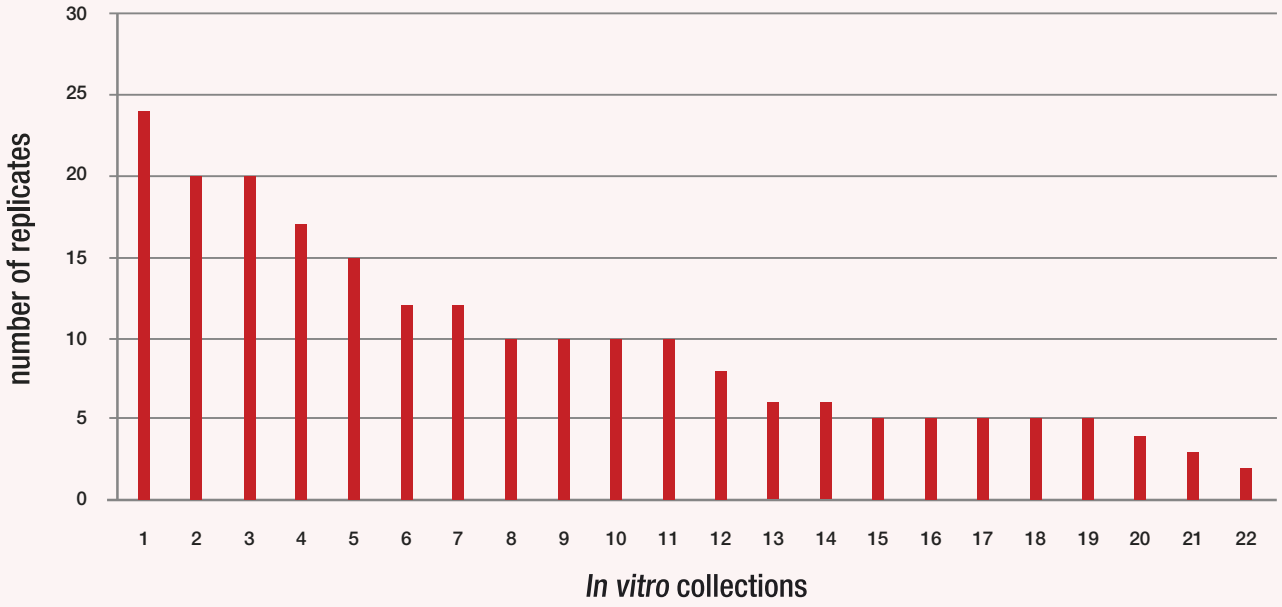
- Number of replicates per accession
- Storage duration between subcultures (*months*)
- Material stored under normal or slow growth conditions
- Storage temperature (°*C*)
- Light conditions, i.e. light regime for the photoperiod

- Light intensity
- Constraints regarding the facilities and the plant materials

Out of the 31 collections, 18 (58%) mentioned having an operation manual containing standard procedures and protocols for the management activities of the *in vitro* collection.

Information provided by 22 of the *in vitro* collections shows that the average number of replicates per accession varies greatly from 2 to 24 plants with an overall average of 10 replicates per accession (see Figure 6.8).

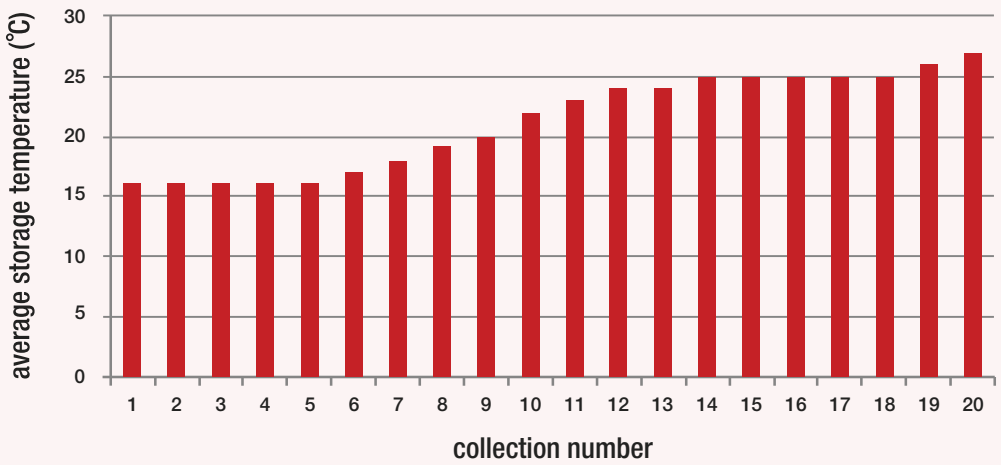
Figure 6.8. *Average numbers of replicates per accession in* in vitro *collections – data from 22 collections.*



The storage duration between sub-cultures also varies greatly from collection to collection from less than one month to 22 months with an overall average of 6 months. The variation in duration is due to the various purposes of multiplication – fewer sub-cultures are needed for conservation purposes while distribution requires more frequent multiplication.

The average storage temperature of *in vitro* collections is 22.5°C. See Figure 6.9 below.

Figure 6.9. *Storage temperature (in °C) of* in vitro *collections – data from 20 collections.*



Out of 21 collections that provided details, 11 confirmed that the material is stored under normal growth conditions, 8 under slow growth conditions and 1 under both. There were no correlations with the size of the collections in the type of growth conditions, i.e. large or small collections are using both conditions. Lower temperatures are more appropriate for medium storage conditions, while higher temperatures are more for micropropagation and distribution and a higher scale.

The most important constraint mentioned regarding *in vitro* plant materials is contamination including from endogenous bacterial infection, requiring frequent sub-culturing. Some accessions or genotypes may be difficult to micro-propagate (mainly ornamentals) and more research and staff is needed to optimize protocols. It was also mentioned that exudate of polyphenol in some accessions led to their low survival rates.

Another major constraint mentioned by 13 out of 20 *in vitro* collections was the lack of continuous power supply and back-up such as a power generator. Limited space available and in many cases no separate storage for *Musa* for the genebanks conserving several other crops was mentioned as a constraint. In addition, there is generally a lack of adequate funding for equipment and skilled staff.

6.1.6 Cryopreservation Collection Management

Cryopreservation is used for the long-term conservation of *in vitro* collections, with minimal probabilities of deteriorating and losses of germplasm. The material is generally not distributed in this form. Two cryopreservation protocols are currently available for a range of banana cultivar groups.

The principles and guidelines for cryopreservation are explained in Panis, B. 2009.

The following 3 institutes maintain germplasm in cryopreservation:

- ITC collection with 938 accessions (76% cultivars, 14 % wild taxa and 9% breeding lines)
- India – NBPGR with 50 accessions
- USDA - Puerto Rico with 10 accessions

And the following additional institutes reported having access to cryopreservation facilities:

1. Australia-DAFF-Mar
2. Brazil -EMBRAPA
3. China-IFTR-GDDAS
4. China-TSFR Lab
5. Ethiopia-EIAR-Jimma-provided by partner institute
6. Ethiopia-EIAR-Melkassa- provided by partner institute
7. Fiji-Sigatoka-provided by SPC
8. Fiji-SPC- but limited only for research
9. India-NRCB-provided by partner institute
10. Nigeria-IITA
11. Uganda-NARO

The objective of the cryobank at the ITC is the safe and long-term storage as a back-up to the *in vitro* collection under medium-term storage. The current number of accessions stored in the cryotank is 873 and the target number of accessions by end of 2016 is 1,313, consisting of present and future accessions in the *in vitro* collection. For more details of cryopreservation of *Musa*, see Section 7.1.2.3.

6.1.7. Conservation of wild species through seed

The feasibility of conserving the wider *Musa* wild diversity through seeds and embryos is being explored. Given the current technologies and available knowledge of the seed biology, seed conservation needs to be researched. Seed- and embryo-cryopreservation offer prospects, but also needs to be tested more widely. Seed storage behaviour is studied as germination of *Musa* seeds in soil is still very unpredictable, takes a long time and germination rate can be very low. Embryo rescue protocols are being used to maximize the regeneration potential.

Priority for conservation would go to the ancestors of the edible bananas for genetic improvement purposes, but in a global collection non-related species could also be considered to serve future needs. Through RTB CRP, an international collaborative programme particularly involving partners in Asia and the Pacific will be set up to investigate the feasibility of seed conservation.

A project for the establishment of a Global *Musa* Seed Bank is included in the Section 6.2 – *Where we want to go*.

6.1.8 Long-term Security of Ex Situ Collections

The long-term safety of genetic diversity is much more likely to be ensured when *in situ* and on-farm conservation is backed up in an *ex situ* collection and when at least two copies of the same accession are conserved in different collections located apart and preferably in different countries and regions, away from threats.

According to the Global Crop Diversity Trust, safety duplication is “the duplication of a genetically identical sub-sample of the accession to mitigate the risk of its partial or total loss caused by natural or man-made catastrophes”. Safety duplication is generally under a ‘black-box’ agreement, i.e. the germplasm is not touched without permission from the depositor and is returned on request. The repository genebank agrees to ensure the long-term conservation and to monitor the viability of the materials.

Any safety duplication arrangement requires a clear signed legal agreement between the depositor and the recipient of the safety duplicate that sets out the responsibilities of the parties and terms and conditions under which the material is maintained. Material sent as *in vitro* or cryo plant cultures may require disease indexing before shipping so that only certified disease-free germplasm is exported, following guidelines on the safe movement of germplasm. A proposed depositor’s agreement that can be customized to meet the individual needs is available from the Crop Genebank Knowledge Base of the CGIAR (CGKB) base at: http://cropgenebank.sgrp.cgiar.org/images/file/forage_grasses/standard_safety_deposit_agreement%202009.pdf

Field genebanks should be duplicated in more than one site and ideally in an *in vitro* genebank as a safety backup. Furthermore, accessions available in other collections may require fewer duplicates in the field.

Despite the over 50 *ex situ* collections worldwide and a global *in vitro* collection at the ITC freely available to any *bona fide* users, there is no robust knowledge of the level of duplicates between and within collections which could inform the need to safety duplicate the most threatened and unique *Musa* material. The picture is complicated by the fact that collections have been lost and later reconstituted more than once, with the high risk of progressive loss in accessions.

Table 6.7. *According to the Global* Musa *Survey, the ITC holds safety duplicates of the following collections and in varying percentages.*

Country	Institutes Acronym	% duplication
Mexico	CICY	90%
Puerto Rico	USDA	90%
Nigeria	IITA	80%
Uganda	IITA	80%
Guadeloupe	CIRAD	75%
Uganda	NARO	65%
Philippines	BPI	58%
Fiji	SPC	57%
Australia	DAFF-SJ	50%
French Polynesia	SDR- PRFC	50%
Papua New Guinea	NARI	50%
South Africa	ARC-ITSC	50%
China	IFTR-GDDAS	45%

Country	Institutes Acronym	% duplication
India	NRCB	35%
Congo DRC	FSK	30%
Ethiopia	EIAR-Jimma	30%
India	KAU	25%
Rwanda	ISAR	20%
Vietnam	FAVRI	16%
French Polynesia	SDR-FPNC	15%
India	NBPGR	14%
Cameroon	CARBAP	11%
Côte d'Ivoire	CNRA	11%
China	TSFR Lab	10%
Philippines	UPLB	10%
Indonesia	ITFRI	5%

When collections have more than 50% of their accessions safety duplicated, on average their material is located in the following (including combinations of cases):

- *in vitro* elsewhere in their own country – 38%
- field collection elsewhere in their own country – 35%
- field collection elsewhere in another country – 36%
- *in vitro* elsewhere in another country, not including ITC - 28%

Of these, 14 institutes have an agreement with another institute for keeping a safety duplication of another *Musa* collection in their field genebank and 13 institutes have a similar agreement in an *in vitro* collection. It was not specified if the institutes managing the safety duplications were located inside or outside of the countries.

In the cases where less than half of the collection is safely duplicated, the main reasons are the following: related to general constraints of funding (for example for BBTV testing), equipment, space such as lab facilities to put accessions *in vitro* prior to safety-duplication and skilled staff. Some mentioned constraints such as i) the necessity to acquire legal permits for germplasm movement, ii) especially to other countries the lack of clear government regulation about duplication of collections outside the country or no national plan for banana, iii) little interest in conservation and collecting of wild species and fertile cultivars for breeding and iv) lack of trust and uncertainties about getting the material back. Some collections have the majority of their materials already coming from another collection or of exotic origin and some collections will only aim at safety-duplicating the national materials of their collections, hence not 100%. Phytosanitary risks have been mentioned. Some accessions may be difficult to conserve through *in vitro*, such as the wild *M. balbisiana* collections. And in some cases, work is needed on characterization and identification before confirming conservation and duplication.

6.1.9. Services associated to ex situ conservation activities

A number of institutes and organizations provide key services to the global system of *Musa* conservation and use, such as molecular characterization, pre-indexing, virus indexing, quarantine services, taxonomic research and breeding programmes, listed in Table 6.8 below.

Table 6.8. *Key services and organizations associated with* ex situ *conservation activities.*

Activity	Organisation
Molecular characterization	• CIRAD, France • IEB, Czech Republic • IITA, Nigeria • University of Cornell • Diversity Arrays Technology, Australia • Beijing Genomics Institute
Virus indexing and quarantine services	• University of Gembloux, Belgium • CIRAD, France • Daff/UQ, Australia • IITA, Nigeria • Plant Protection Research Institute (PPRI) South Africa
Major breeding programmes	• CARBAP, Cameroon • CIRAD, France (Guadeloupe) • EMBRAPA, Brazil • Fundación Hondureña de Investigación Agrícola (FHIA), Honduras • IITA, Nigeria and Uganda • NRCB, India

6.1.10. Collecting and acquisition of materials and gap filling

The Global *Musa* Survey aimed to obtain an inventory of the main institutes involved in the conservation of *Musa* diversity as a first step. As an immediate follow-up to the survey, Bioversity is contacting the respondents to discuss the possibilities of including information of each accession on MGIS, by signing a Data Sharing Agreement (DSA) to ensure that the ownership, attribution and responsibility for the data remains with the data provider. The DSA spells out clear terms and conditions for the use of the data. It would allow for an update of old accessions lists in MGIS and for specific diversity analyses to be carried out.

Therefore the survey did not allow for a complete identification of the material conserved in each of the genebanks as this can only be assessed by analysing the full list of accessions, passport and characterization data.

The key constraints in understanding the total *Musa* diversity for collecting and gap filling, according to the MusaNet Diversity Thematic Group, are the following:

- Adapted characterization for wild specimens
- Insufficient morphological characterization in many cases
- Insufficient molecular characterization in most cases
- Wild specimens recalcitrant for *ex situ* conservation

- Unreliable *in situ* conservation of cultivars
- Lack of *ex situ* collections: wild accessions, cultivar subgroups, typical Indian and Philippines germplasm and Fe'i banana

The curators however provided feedback on the gaps to fill in their own collections to fulfil their objectives (species, geographic area, traits). In some cases, gaps in germplasm with potential for resistance or tolerance to specific pest and diseases of national or regional importance were mentioned. Gaps in diversity within the regions of the collections were also mentioned (e.g. gaps in plantain diversity from West Africa). Some institutes also identified gaps of specific types of accessions, including wild species, plantains, diploids, disease resistant cultivars, etc. and provided details of the poorly represented diversity and where further collecting is needed.

Furthermore, some large collections of regional significance lack a representative spectrum of wild taxa. Many of these taxa are also still missing in the ITC. This seriously hampers comparative taxonomic research as well as the search for potentially promising traits (e.g. for breeding). A representative set should be selected, and at least introduced into regional collections. In the regions where taxa are growing in the wild, their duplicates should be maintained in the national collections.

Regarding the ITC, the main gap in cultivars is of Indian germplasm (edible and BB *Musa*). The germplasm has been collected but is not represented in the ITC and therefore not yet available for wider distribution and use. This is further discussed in Chapter 7 – *The ITC Global Musa Collection*.

In many cases, cultivars subgroups are collected nationally but not adequately classified. In other cases, they have not yet been internationally assessed. In national genebanks, at least 25 individuals of each *M. acuminata* subspecies from a wide geographic range are needed.

The MusaNet/Trust Bogor meeting in 2012 also acknowledged that the diversity maintained in *ex situ* collections is not fully representing the diversity maintained on-farm and in the wild in most countries. This is mainly due to:

- Lack of ecologically stratified collecting missions
- The purpose of most collections is research and support to breeding and not specifically the conservation of diversity
- Absence of complete national lists of existing cultivars and wild species
- Lack of systematic prospection and of collecting missions exploring remote areas as collecting missions tend in general to take place in areas accessible by roads.

A description of recent collecting activities was provided by 32 collections and is detailed in Table D.5 in Annex D.

MusaNet raised funds to organise two collecting missions in Indonesia in 2012 and 2013. The first mission took place in North Sulawesi at the area of Gunung Ambang and Bogani Nani National Park. Twenty-six accessions have been characterized including 11 wild species (*Musa acuminata* and *Musa lolodensis*) and 15 cultivars. The second mission took place in Ambon and Seram Island (Masohi, Manusela, Wahai and Kairatu). Thirteen accessions have been characterized including five wild *Musa acuminata* and eight cultivars. Fresh cigar leaves have been sent to the MGC for ploidy and molecular analysis (see Section 1.3.1.1 for more details).

Germplasm collected during past missions is being GIS mapped. This exercise highlights two important issues to be resolved. The first issue is the quality of the data collected along with the germplasm: exact collecting locations are not always well-documented and status of accessions, i.e. wild or cultivated, is not always adequately identified. Additionally, the use of the term "wild" is sometimes erroneous as quite

often seeded cultivated accessions are registered as "wild" even when collected within home gardens. The second issue is the traceability of accessions from the collecting missions to *ex situ* collections.

The Global Crop Diversity Trust is partnering with Bioversity International and the Centro Internacional de Agricultura Tropical (CIAT) to identify gaps in collections of major crops worldwide, including *Musa*, focusing on wild species. The goal is to develop a methodology to identify collecting priorities using priority maps, databases and other information. The proposed methodology has been published for a number of seed crops using *Phaseolus* bean as a model. Analyses for cultivars of *Musa* are also under development by CIAT researchers and others.

SECTION 6.2 *MUSA COLLECTIONS AROUND THE WORLD* - WHERE WE WANT TO GO

One of the objectives of this Strategy is to conserve the entire *Musa* genepool in perpetuity by a network of well-managed efficient and effective *ex situ* collections and make available the resources for research and improvement. This can be achieved by the following specific outputs:

1. Comprehensive assessment of the content of *Musa ex situ* collections towards understanding the gaps and priorities.
2. Improved effective management of *ex situ* collections and enhancement of services leading to cost-effective conservation of the genepool.
3. Identification and setting up of a global network of partners with specific responsibilities for conservation of the *Musa* genepool including the safety duplication.
4. Increased access and targeted use of the ITC collection in partnership with regional and national collections.

6.2.1 Major needs and priorities

The feedback from the Global *Musa* Survey suggests that funding and capacity building are the most urgent needs for improving the current situation in the collections, particularly for ensuring the collecting of threatened materials and for research on the germplasm. See details in Table 6.9 below.

Table 6.9. *Most urgent needs of the collections regarding funding, facilities and staff.*

	Inadequate	Adequate	Very good
Funding for routine operations and maintenance	60%	32%	8%
Number of trained staff	61%	35%	4%
Status of buildings, facilities and equipment	45%	43%	11%
Funding for collecting germplasm	76%	20%	4%
Funding for research on the collection	70%	28%	2%
Level of use by breeders, researchers or growers	52%	33%	15%

The need for adequate and continuous financial support has been mentioned by 89% of the curators and the following priorities were specified:

Assessment of the Diversity and Gap Filling:

- Characterization and evaluation of germplasm; new germplasm collecting; routine germplasm management operations; research on seed conservation (of wild accessions).

Effective Management:

- Long-term maintenance of field, greenhouse and *in vitro* conservation; frequent regeneration; safety duplication of the collection; dealing with new emerging diseases and climate change.

Distribution of germplasm:

- Mass multiplication including replanting of cultivars after natural disasters; creation of awareness both locally and at international levels; research on germplasm to increase use. This is discussed further in Chapter 10 - *Distribution and Safe Exchange of Germplasm*.

All these can be strengthened by solid partnerships at the regional and global level and are discussed in more details the sections below.

6.2.2 Assessment of the Diversity in Ex Situ Collections and Gap Filling

- A comprehensive assessment of the content of *Musa ex situ* collections towards understanding the gaps and priorities would entail the following activities and steps:
- Surveying all *ex situ* collections to obtain a complete list of the specific accessions conserved with taxonomic identification and origin to create an inventory of what is currently in *ex situ* conservation. This should be led by MGIS.
- Analysing of the collated information serving as a basis for the identification of gaps and redundancy and recommendations for improving the conservation and use of collections.
- Making the inventory available to the *Musa* community through MGIS.
- Analysing the priorities for filling in the gaps in diversity and making recommendations for developing targeted collecting missions and seeking funding.
- Considering hot spots of *Musa* diversity for *in situ* conservation.

6.2.2.1 Documentation of information and knowledge

The assessment of the complete diversity will require that all *ex situ* collections participate in the completion of MGIS by providing passport, characterization and evaluation data available on all germplasm currently maintained *ex situ*. This includes the harmonization of databases and training of curators on data and database management. It will facilitate the exchange of knowledge on the conservation of *Musa*, the possibility to develop catalogues and raising public awareness on the current conservation and the threats to *Musa* diversity.

Technical guidelines for documentation of collections are available and the three recent MusaNet workshops (Guadeloupe 2013, Trichy 2014 and Cameroon 2015) provided guidance on all the documentation aspects including taking photos. In addition, the regional workshop carried out at Cameroon in May 2015 included training on tools such as a mobile device to facilitate the field recording and characterization of *Musa* germplasm. This is discussed in more detail in Chapter 9 - *Information Management*.

6.2.2.2 Filling in the gaps in diversity

A number of recommendations have been formulated from the survey as well as during the MusaNet consultations, including the workshop in Bogor in 2012. It was stressed that national and international research institutes need to work together to ensure the long-term safe conservation of *Musa* genetic resources.

The 2012 Bogor meeting suggested that the geographic structuring of genetic diversity should be studied to fill in the gaps of knowledge on the most valued diversity for breeding. Molecular approaches for resolving taxonomic issues needs to be combined with classical morphological studies, as molecular markers have limitations. For example, they can assign accessions to a group and/or subgroup but cannot resolve the redundancy problem of identification, as differences between phenotypes may not show up in genotypes.

MUSA WILD SPECIES DIVERSITY

It was suggested that all wild species be conserved in the national collections of the country of origin when they exist or regional collections, preferably in the field to avoid somaclonal variations. A representative of all wild species should also be made available for exchange through the ITC.

However, one issue that should be carefully addressed is the safe movement of wild species from where they naturally occur to other parts of the world as they may become weeds in a new environment with significant negative ecological impacts and potentially proliferate banana pests and diseases. This also can make banana disease eradication more complicated because of the difficulties in eradicating the host, necessitating repeat visits over many years to exhaust the seed reserve in soil.

A short-term strategy for capturing the *Musa wild species* diversity of particular value for breeding would focus on *M. acuminata* and *M. balbisiana* with special attention for pest and disease resistance and for favourable agronomic features. A long-term strategy would include species other that *M. acuminata* and *M. balbisiana* in the Eumusa and Rhodochlamys sections and beyond (e.g. sections Australimusa and Callimusa). The materials should be screened for disease resistance along with parthenocarpy and sterility alleles.

EDIBLE DIPLOIDS AND TRIPLOIDS

As the vast majority of edible diploids and triploids have been explored, the accessions should also be revisited for fruit quality and good agronomic traits, such as disease resistance or higher yield. Available passport data and local knowledge should be examined.

6.2.2.3 Collecting the missing diversity in the wild and in villages

Collecting priorities for non-explored diversity, i.e. subspecies and cultivars not currently in any *ex situ* collections, would include collecting in the wild and in villages. These areas may contain cultivars as well as wild (endogenous or naturalized) germplasm. This is further discussed in Sections 4.2.1 and 4.2.2.

The case of the Fe'i group is important to mention here as it is a special case. In the Pacific, great progress has recently been made in the collection of numerous cultivars belonging to the subgroups Maoli-Popo'ulu and Iholena, and to the Fe'i group. The Fe'i banana, a group of Australimusa cultivated derivatives, was until recently, disappearing. Recent efforts have succeeded in safeguarding several cultivars, and the diversity of this group is for the first time the subject of concerted taxonomic investigation. Further collecting in the Lousiade Archipelago in the Pacific and nearby areas would be fruitful. While several islands seem to have been sufficiently explored, others may contain more cultivars, especially in the more remote parts (e.g. mountainous areas). This operation is ongoing, but it currently depends on *ad hoc* financial support, and therefore concerted planning of further explorations would be useful.

There is also a great need to eliminate BBTV in several Pacific Island nations. There are a few unusual Maoli-Popo'ulu-like cultivars in Pohnpei, Federated States of Micronesia, that need collecting and assessing. Tongan Maoli /Popoulu diversity needs collecting and freeing of Bunchy Top (Jeff Daniells pers. com.). A joint project with the SPC was developed in 2007 to address this issue but was not successfully funded.

It is suggested that creating national registries of farmers' cultivars and wild species would be a first step to understanding what is conserved and what gaps may need to be filled. But dealing with homonyms and synonyms is likely to be extremely complex in the case of farmers' cultivars and therefore difficult to apply in many countries.

Increasing collaboration through MusaNet will help in assessing what is conserved and accessible for use and priorities for collecting.

From the Global *Musa* Survey, 37 collections provided a description of their future collecting plans.

6.2.2.4 Guidelines for Collecting

Recommendations from the MusaNet/Trust Bogor meeting for the collecting methodology for wild species and cultivated materials are the following:

WILD SPECIES

- Ensure that seeds are collected in addition to suckers.
- Between 2,000 and 10,000 seeds per genotype/accession should be collected.
- Seeds should be sampled from the whole population rather than from a small amount of individuals.
- Suckers should be sampled within a given population by either random sampling or sampling of selected individuals exhibiting interesting characteristics.
- Leaf sampling for DNA extraction is required with systematic grid sampling of populations for population genetics analysis.
- Herbarium specimens should be collected.
- Passport data should be documented.
- Photos of specific characters should be taken.
- Common names of wild specimens should be recorded along with the associated local knowledge.
- Collected materials should be deposited in the most relevant national collections and in the ITC for global distribution. A sample could also be provided to botanic gardens.
- Seeds should be conserved as a complementary strategy to conserving the clones.
- Genebank experts from other species should be consulted to design strategies to adequately represent genepools in target collection areas.

CULTIVATED MATERIALS

- If collecting for breeding, the traits to focus on are low height, bunch shape, number of fruits, resistance to diseases and diploidy.
- If collecting for conservation purposes, the methodology should focus within each village for systematic leaf-sampling for DNA extraction and rationalized sucker-sampling for conservation.
- Photos should be used to describe the plant since it might be difficult to obtain plants at the right stage of development.
- Collected materials should also be deposited in a national collection and in the ITC for global distribution.
- A short socio-economic description of the village should be provided (e.g. accessibility or distance from important roads and urban centres).
- A discussion with the farmers should provide additional information and genders should be differentiated for interviews, such as:
  - How long has the cultivar been there?
  - What is special about the cultivar?
  - What parts of the plants are used and how (in addition to the fruits)?
  - What is the frequency of planting, e.g. common, rare or marginally planted in the village?

The knowledge necessary for collecting and conserving priority materials is the following:

- Genetic structure
- Distribution areas (per taxon)
- Populations density within their distribution areas
- Population dynamics and ecology
- Threats (e.g. habitat loss, climate change)

Developing an electronic collecting form linked to MGIS has been proposed to directly rationalize the sampling and avoid redundancy in collections. It has also been suggested that taxonomy experts in the local *Musa* community be part of the collecting team.

Collecting of wild species should not focus on identifying traits as this occurs later during the characterization and evaluation of the ex situ collections. All new wild resources need to be duly observed in the field so that this information can be submitted to taxonomy experts and curators for the final assessment, description and determination of botanical status. Only then can the accessions be virus indexed and made publicly available from the ITC.

6.2.3 Improving Effective Management of Ex Situ Collections

The effective management of *ex situ* collections and enhancement of services leading to cost-effective conservation of the genepool should include the following activities:

- Develop and update quality standards for *Musa* genetic resources conservation management
- Create a platform for effective information exchange and sharing of methods, techniques and experiences between collection managers
- Train and build capacity in the use of methodologies, standards and best practices that contribute to improved management of the collections

6.2.3.1 Development of guidelines and standards

The MusaNet workshop in 2013 discussed the main gaps in bringing up the capacity of the collection management and what is needed at the local/national, regional and global level. There is a need for specific guidelines to be developed for:

- Field management including specific information on topics such as wild species management and ecological regions
- Collecting and acquiring new materials on missions in a collection
- Seed bank management
- Data management and use of the descriptors

FIELD MANAGEMENT GUIDELINES FOR GERMPLASM COLLECTIONS

Field genebanks generally require more labour, inputs and space (land) than other methods of conservation. They are also faced with higher levels of risk from natural disasters and adverse environmental conditions like drought, floods or attacks from pests and diseases. When field genebank conservation is the only viable alternative, careful planning and field management can help to mitigate the risks. Field genebanks should be duplicated in more than one site or in an *in vitro* genebank or cryobank as a safety backup.

MusaNet organized two workshops with the objective of discussing the most urgent needs of *Musa* collection curators vis à vis the management of the germplasm and its associated information. They involved the 13 partners of the TRC who also manage some of the most important national and regional collections. The first workshop was in Guadeloupe hosted by CIRAD in December 2013 and the

second one was in India hosted by NRCB in December 2014. They focused on discussion and making recommendations for ensuring the correct identification of the materials conserved and making this information available to all users. But they also included discussions on the field management of the accessions and best practices for the conservation and documentation of *Musa* germplasm, including the safe-movement of materials.

A similar, but regional, MusaNet workshop for West and Central Africa was held in 2015 at the CARBAP field collection in Cameroon. During this workshop, field management was discussed during a field session and classroom session. Topics covered include optimal environmental conditions, planting preparation and methods, fertilization, and weed and disease control. Another regional MusaNet workshop will be held late 2016 in Uganda, focusing on characterization of the EAHB subgroup.

The MusaNet workshops recommended that the 2008 Regeneration Guidelines be updated and a new set of technical guidelines be developed for the full range of activities in field management including tissue culture establishment. This will be coordinated by the MusaNet Conservation Thematic Group.

Musa-specific field management guidelines should be developed and cover all the following practices: planting plan; planting material; climatic/environmental hazards; cover crop management practices; pest and disease management; monitoring; regeneration; tissue culture and establishment in field; health testing; back up of the collection; data recording and management; financial and human resources; logistics; and vandalism control.

The information on health testing should include the following: efficient virus cleaning protocol to produce clean germplasm; maintaining field collections free of viruses; spread of diseases like BBrMV; adequate measures or actions to reduce abiotic and biotic stresses particularly for the viral diseases are extremely needed; biotic and abiotic factors are major threats; *in vitro* conservation to save accessions from various diseases; need for guidelines and research of uniform plants within a single cultivar; and more regular screening of accessions of the deadly viruses (BWX, BBTV etc.) should be carried out.

It is important that revised guidelines include practices that ensure the objective of the field collection management and that these are clearly identified. The field sites need to be planned including initial trials to test treatments and management requirements. The initial trial plan can be circulated for input from experts, supervisors, and others with relevant knowledge. The MusaNet networks of curators can be used for review. Most importantly, all activities should be accompanied by documentation of the changes made, providing a valuable record supplemented with photos. The funds need to be adequate to meet the objectives.

There is a need to develop safety duplication options. It is proposed that each field genebank should be working very closely with and be backed up by an *in vitro* collection.

6.2.3.2 Development of a Global Musa Seed Bank

A Global *Musa* Seed Bank proposal is being developed and would aim to conserve and distribute seeds under the International Treaty on Plant Genetics Resources for Food and Agriculture (ITPGRFA).

The first step before seeds are collected is to assess the diversity within/between populations, to make decisions on how many seeds to collect so that they can be conserved and distributed. The proposal is to first analyze seed lots of *M. acuminata* and *M. balbisiana* growing in a clearly delineated distribution range such as islands. DNA will be extracted from seeds as well as from the mother plants to determine the genetic variation within a population.

It is proposed that priority should be given to *M. acuminata* (10 -20 accessions per subspecies), with fewer accessions for other *Musa spp* (of which more than 10 species should be collected in Indonesia, Malaysia, N. Burma, Bhutan, or SW China and Vietnam in the framework of the Crop Wild Relative Project (CWR) of the GCDT). Agreements will be made with the various contacts via MusaNet.

Germination of *Musa* seeds in soil is still unpredictable, takes a long time and germination rates are

often very low. To partially solve this problem, embryo rescue protocols were developed by Bioversity/ KU Leuven. When linked to chemical viability test (tetrazolium chloride TTC) it was observed that embryo rescue increases the regeneration potential. However soil germination needs to be further investigated. Germination could be tested in Meise Botanic Gardens in Belgium, where some wild bananas (preferably *M. acuminata* and *M. balbisiana* but not exclusively) could be grown in the greenhouses under controlled conditions and seed harvested at different times.

Currently storage behaviour at room temperature, 5°C, -20°C and -196°C is tested. While clear conclusions on population genetics are reached, recommended storage and germination methods should be followed and seeds should preferably be produced in a disease-free environment. Research is also needed on the transmission of viruses in seed.

6.2.3.3 Capacity building

Ensuring that adequate skills and equipment including infrastructure are available for the range of germplasm conservation activities has been mentioned as a priority by 51% of the collection managers in the Global *Musa* Survey. Particularly important is the need for full time curators and stable teams including breeders to ensure continuity of the collections. Equipment is needed, in particular for *in vitro* and cryopreservation labs, as well the maintenance of screenhouses to back up field collections.

It is important to strengthen the regional networks by organising national and regional workshops and stimulating academic exchange among national, regional and international centres and genebanks. It would also be helpful to develop academic training in plant genetic resources management and increase capacity building for virus indexing and molecular characterization.

It would be beneficial to improve information technology capacity (software and hardware) and ensure the completeness of MGIS with data on all available germplasm. It is imperative that the *Musa* 1996 descriptor book be updated. This subject is discussed in Chapter 5 on *Characterization* and Chapter 9 on *Information Management*.

Actions are needed to address BSV and its constraints for germplasm exchange and policies need to be strengthened for *Fusarium* quarantine restrictions - see Chapter 10 on *Distribution and Safe Exchange of Germplasm*.

6.2.4 Global Partnerships for the Safeguard of the *Musa* Genepool

A global network of partners with specific responsibilities for conservation of the *Musa* genepool including the safety duplication needs to be optimised and strengthened. This would entail the following activities:

- Establish global partnerships and collaboration in conservation of collections.
- Improving the documentation of collection through standard data gathering and verification of passport data, phenotypic, cytological, and genotypic characterization.
- Field verification and morpho-taxonomic characterization, flow cytometric ploidy determination and SSR characterization of the ITC collection.
- Identify and eliminate duplicates and redundancy in and across collections based on the improved documentation of accessions.
- Introduce missing diversity to ensure full coverage of priority genepools at the national, regional and global level.
- Increase the safety duplication of unique accessions in genebanks by using at least two conservation methods (e.g. in field, *in vitro*, cryopreservation) and at least in two different locations.
- Develop priority lists of accessions (core sets) for specific collections (national, regional, global) for long-term conservation.

- Identify candidate accessions for the global core collection at ITC.
- Establish partnership agreements with regional and national field collections for complementary responsibility sharing to preserve global core accessions.
- Duplicate the global core accessions *in vitro* and in cryopreservation at the ITC.
- Ensure the continued safety back up of the ITC cryopreserved collection.

There is an urgent need to set up more locations of international field planting or *in vitro* culture conservation, which will be safer for germplasm duplication and more effective for *Musa* distribution. All national germplasm collections should be safely duplicated at the global level.

It is essential to ensure the maximum possible availability of the materials at the ITC for sharing as promising cultivars are identified.

The improved characterization (phenotyping and genotyping) of germplasm in all collections will provide information to allow curators to make more informed and effective decisions on the rationalization of their own accessions. At the national, regional and global level, this will also provide the means to identify core sets of accessions for long-term conservation, which will embody the entirety of *Musa* diversity. At the global level, the data from the field verification and molecular characterization of ITC accessions will provide morphological and molecular references, which collections worldwide will be able to apply to their own accessions and studies. From this, ways to reduce duplication of material in collections needs to be developed, core collections created and further collecting priorities defined. The regeneration and field verification project can be used as starting point for further research on maintaining genetic integrity and avoiding 'off-types' in *in vitro* collections.

The contexts in which collections in banana-producing regions function are very different. The Latin America and Caribbean region is remarkable for large historical collections predominantly used for breeding and the absence of significant indigenous diversity. Sub-Saharan Africa represents a secondary centre of diversity with very few adequately-resourced national collections but four important regional collections: NARO and IRAZ in East Africa and CARBAP and IITA in West Africa. There are numerous national collections in Asia and the Pacific which conserve unique indigenous diversity.

While no single model of national and international collections can be superimposed on all of the regions, the broad roles of collections at national, regional, international and global levels are broadly described below (from INIBAP 2006).

Role of National collections:

- Collecting and conserving diversity at national level
- Field and/or *in vitro* conservation of indigenous accessions
- Characterization and evaluation
- Participating in MGIS and information management training
- National disseminating of germplasm especially to farmers
- Expertise and capacity building in production, use and local cultivars

Role of Regional and internationally-recognised collections:

- Conserving the diversity at the regional level
- Field and/or *in vitro* conservation
- Characterization and evaluation to facilitate breeding strategies
- Participating in MGIS and information management training

- Dissemination of germplasm
- Expertise and capacity building in taxonomy, germplasm management, virus indexing and multiplication technologies

Role of the Global Collection at ITC:

- Maintaining FAO "in trust" collection
- Cryopreservation and *in vitro* conservation
- Processing germplasm for virus-indexing and sanitation when necessary
- Coordinating and upgrading MGIS
- Participating in MGIS and information management training
- International dissemination of germplasm especially to collections, researchers and breeders
- Expertise and capacity building in conservation technologies and germplasm management

Some institutes provide additional key services such as:

- Virus pre-indexing
- Virus therapy
- Virus-indexing
- Ploidy verification
- Genotyping

The establishment of a global reference field collection integrating subsets that represent specific parts of the diversity and are held by different collections is strongly recommended. This concept should stimulate collaboration amongst collections within and across regions and will require the sharing of conservation responsibilities with the commitment to function as (partial) safety back-up of the global core set. In this respect, it is crucial that participating field collections receive the necessary support in the long term, that through partnerships they actively contribute and benefit from:

- shared quality standards (implementation of the field genebank guidelines),
- information exchange on holdings (through the creation of a platform for information exchange between collections), and
- training and assistance in conservation technologies and germplasm management, particularly on pest and disease management.

In order to motivate collections, research and service partners to take up their role in the global system, a clear need was expressed by MusaNet members for creating incentives for participation.

The major benefit that is expected to be generated in a well-functioning system is a greater access to information and materials. Material and information providers should be also able to benefit from training and capacity building, be priority partners in projects and wherever possible, be duly acknowledged, particularly in publications.

Data provision should be made part of projects in order to ensure that it is actually done.

As common methodology and quality standards tighten the network, this would form a more favourable basis for attracting funds and government commitment to support conservation activities.

Incentives may also include increased resource sharing, such as sharing of health testing and molecular characterization services in support of conservation and exchange activities.

SECTION 6.3 – *MUSA COLLECTIONS AROUND THE WORLD* – HOW WE WILL GET THERE

The aim of the global system for *ex situ* conservation is to conserve the entire *Musa* genepool in perpetuity and promote the safe exchange and use of a wide range of diverse germplasm by a network of well-managed rationalized collections. Many of the proposed actions below are also found in other chapters of the Strategy.

Table 6.10. *Proposed objectives and actions regarding* Musa *collections around the world.*

Objective	Proposed Actions
1. Comprehensive assessment of the content of *Musa ex situ* collections towards understanding the gaps and priorities.	• Survey all *ex situ* collections for a complete list of accessions conserved with taxonomic identification and origin • Make the inventory available to the *Musa* community through MGIS • Analyze priorities for filling gaps in diversity and make recommendations for targeted collecting missions • All *ex situ* collections to participate in the completion of MGIS by providing passport, characterization and evaluation data on germplasm maintained *ex situ* • Harmonize databases and train curators on data and database management • Develop catalogues on current conservation and threats to *Musa* diversity • Develop technical guidelines and training on collection documentation to facilitate field recording and characterization • Conduct geno-geography research to fill gaps of knowledge on the most valued diversity for breeding • Combine molecular approaches for resolving taxonomic issues with classical morphological studies • Conserve all wild species in country of origin national or regional collections and in the field to avoid somaclonal variations • Make available representatives of all wild species for exchange through the ITC • Address the safe movement of wild species carefully from where they naturally occur to other parts of the world • Develop a short-term strategy for capturing the *Musa* wild species diversity for breeding focusing on *M. acuminata malaccensis* derived AA cultivars and BSV-free *M. balbisiana* • Study cultivars of *M. acuminata* subspecies for Fusarium resistance and production potential • Develop a long-term strategy for species other than *M. acuminata* and *M. balbisiana* in the Eumusa and Rhodochlamys sections and beyond (e.g. sections Australimusa and Callimusa) • Revisit edible diploids and triploids accessions for fruit quality and good agronomic traits, such as disease resistance or higher yield • Further collecting of the Fe'i banana, in the Lousiade Archipelago and nearby • Create national registries of farmers' cultivars and wild species to understand what is conserved and the gaps • Fill in the gaps mentioned by specific countries and accessions made available to the ITC for worldwide distribution • Develop an electronic collecting form linked to MGIS • Observe all new wild resources in the field so that information can be submitted to taxonomy experts and curators for final assessment, description and determination of botanical status.
2. Improved effective management of *ex situ* collections and enhancement of services leading to cost-effective conservation of the genepool.	• Develop and update quality standards for *Musa* genetic resources conservation management • Create a platform for effective information exchange and sharing of methods, techniques and experiences between collection managers • Train and build capacity in the use of methodologies, standards and best practices that contribute to improved management of the collections • Update the 2008 Regeneration Guidelines • Develop minimum descriptor lists for the various subgroups – including EAHBs, an output of the regional MusaNet workshop that will be held in late 2016 in Uganda

Objective	Proposed Actions
	• Develop field management guidelines including specific information on groups such as wild species management and ecological regions • Develop a new set of technical guidelines for the full range of activities in field management including tissue culture establishment • Develop safety duplication options such as genebank working closely with and backed up by an *in vitro* collection • Develop guidelines on collecting and acquiring new materials on missions in a collection • Develop seed bank management guidelines • Develop guidelines on data management and use of the descriptors • Assess the diversity within/between populations, to make decisions on how many seeds to collect so that they can be conserved and distributed • Develop a Global *Musa* Seed Bank to conserve and distribute seeds under the ITPGRFA • Ensure adequate skills and equipment including infrastructure available for the range of germplasm conservation activities • Strengthen regional networks by organising national and regional workshops and stimulating academic exchange among national, regional and international centres and genebanks • Develop academic training in plant genetic resources management and increase capacity building for virus indexing and molecular characterization • Improve information technology capacity (software and hardware) and ensure completeness of MGIS with data on all available germplasm.
3. Identification and setting up of a global network of partners with specific responsibilities for conservation of the *Musa genepool* including the safety-duplication.	• Strengthen the global network of partners with specific responsibilities for conservation of the *Musa* genepool including the safety duplication • Improve characterization (phenotyping and genotyping) of germplasm in all collections to allow curators to make decisions on rationalization of accessions • Introduce missing diversity to ensure full coverage at national, regional and global level • Identify core sets of accessions for long-term conservation, which will embody the entirety of *Musa* diversity • Field verification and morpho-taxonomical characterization, flow cytometric ploidy determination and SSR characterization of the ITC collection • Increase safety duplication of unique accessions in genebanks • Set up more locations of international field planting or *in vitro* culture conservation, safer for germplasm duplication and effective for *Musa* distribution • Rationalise national collections based on improved characterization (phenotyping and genotyping) of germplasm • Develop priority lists of accessions (core sets) for specific collections (national, regional, global) for long-term conservation • Duplicate the global core accessions *in vitro* and in cryopreservation at the ITC • Establish a global reference field collection integrating subsets that represent specific parts of the diversity held by different collections • Support the sharing of resources, such as health testing and molecular characterization services in support of conservation and exchange activities.

CHAPTER 7.
THE ITC GLOBAL *MUSA* COLLECTION

SECTION 7.1 THE ITC GLOBAL *MUSA* COLLECTION - WHERE WE ARE NOW

The Global *Musa* Germplasm Collection of the International Transit Centre (ITC), based at the Katholieke Universiteit Leuven (KULeuven), Belgium, was set up in 1985. The collection is held 'in trust' under the auspices of FAO and managed by Bioversity International. The ITC is the largest collection of *Musa* germplasm with around 1,500 accessions (See Table 7.1).

The objectives of the ITC are the following:

- Providing long-term and sustainable conservation of *Musa* genetic resources.
- Maintaining a source of genetic diversity and related information in the public domain.
- Contributing to understanding *Musa* diversity through characterization.
- Providing a service for the safe movement of germplasm and related information.
- Developing and transferring *ex situ* conservation technologies.

At the global level, the ITC collection functions effectively in assuring medium and long-term conservation of the broadest range of *Musa* diversity. Strongly linked with MusaNet, the ITC operates via a network of field collections, exchanging germplasm and providing a back-up service. ITC has strong relationships with regional collections in Asia, Africa and Latin America and with several national collections providing an essential support role to the long-term conservation of the global collection. The ITC is closely linked with these partner collections and cooperates in terms of research and capacity building in conservation methods.

Many research institutes are partnering with the ITC in the areas of health testing (DAFF/University of Queensland in Australia, University of Liège at Gembloux in Belgium and CIRAD), and characterization of the collection (IEB, CIRAD), cryopreservation research (KULeuven) and several additional national collections are involved in field verification of the identity of the ITC accessions (see Section 7.1.2 – *Management of the Collection at the ITC*). The ITC collaborates closely with the Institut de Recherche pour le Développement (IRD) in Montpellier, France, for safety duplication of its accessions.

The ITC has a key role as provider of samples of the widest available range of *Musa* diversity that is guaranteed clean of pests and diseases to researchers, collections and breeders.

The ITC is actively developing best practices in genebank management such as germplasm acquisition, health testing, medium-term conservation, cryopreservation for long-term storage, lyophilized leaves and DNA banking, data management, monitoring genetic integrity, distributing germplasm, and sharing this expertise with collection curators and researchers around the world.

The ITC is funded by the Genebanks CRP and the Global Crop Diversity Trust (GCDT), which provide security in the core operations of the genebanks and work towards improving individual performance standards and strengthening quality and risk management systems. The CGIAR sets performance targets for all genebanks which cover topics such as germplasm acquisition, availability, security, QMS and cost efficiency.

A review of the ITC was conducted in 2010 to assess the performance of the ITC in terms of the conservation and distribution of *Musa* germplasm and included an evaluation of the service by users. Furthermore, the ITC was externally reviewed in 2013 by two reviewers designated by the GCDT, within the framework of the Genebanks CRP. The resulting Recommended Action Plan included improvements to be made, which are included in this chapter.

7.1.1 The Content of the ITC

Over the years, *Musa* genetic resources have been acquired by the ITC from 52 donor sources in 37 countries, including major field collections, collecting missions in the crop centres of origin and diversity, and from banana breeding programmes worldwide (see Table 7.1 below).

The ITC collection is comprised mainly of cultivated bananas (75%), and to a lesser extent of wild *Musa* species (16%), and of improved cultivars (9%). Of the entire collection, 9 accessions are of *Musa textilis* and *Ensete* spp., both not included in Annex 1 of the ITPGRFA but available to all *bona fide* users as they are included in its article 15b concerning the materials in the international collections of the CGIAR.

In recent years, the representation of the *Musa* genepool in the ITC collection has improved with the acquisition of 250 accessions from priority collections supported by the GCDT.

Table 7.1. *Number of accessions at ITC by source and type of genotype, dated December 2014.*

Type of source	Donor	Accessions	Genotypes
Major Field collections	FHIA, Honduras (1988)	97	Wild/cultivated forms
	IITA, Nigeria (1986-1987)	85	AAB-plantain
	IRAZ, Burundi (1987)	54	EA-Highland bananas
	CIRAD, Guadeloupe (1987-1990)	267	Wild/cultivated forms
	CARBAP, Cameroon (2010)	41	AAB-plantain
	NRCB, India (2010-2011)	57	AB, AAB and ABBs
Collecting missions	Papua New Guinea (1989-1990)	278	Diploid wild/cultivated
	Vietnam (1996)	43	Wild/cultivated forms
	Tanzania (2002-2005)	56	EA-highland bananas
	DR Congo (2005)	38	Semi-dwarf AAB-plantains
Breeding programmes	CARBAP, CIRAD, EMBRAPA, FHIA, IAEA, IITA, INIVIT, TBRI	126	Improved high yielding and disease resistant cultivars
Others	Other collections, botanical gardens, private persons	337	Wild/cultivated forms
TOTAL		**1,479**	

7.1.1.1 Gaps at the ITC and acquisition strategy

The ITC collection has a good overall representation of cultivated bananas including plantains. However some specific cultivated groups and subgroups from some geographical areas as well as wild species are still under- or unrepresented in the collection. Future acquisition should focus on obtaining unique representatives of these species and cultivated groups.

There are still an estimated 300 to 400 cultivars and wild specimens known to be missing in the collection (Rony Swennen, pers. comm.) and the TAG meeting in 2008 discussed the potential sources of germplasm to fill the gaps at the ITC (see Table 7.2).

From a utilization perspective, interesting materials with potential or known breeding and research value should also be prioritized for acquisition: tolerance to abiotic stresses (e.g. drought, salinity) or disease

resistance traits (e.g. BBTV, BXW, *Foc* TR4). Some specific cultivars with direct users' interest (e.g. Budless Kepok and Dwarf Pisang Awak, Dwarf Silk, Lowgate, and Lakatan and Saba from the Philippines) have also been targeted for acquisition by the ITC.

Table 7.2. *Wild species and genotypes missing from the ITC and their potential collecting and donor sources (based on information from TAG 2008 meeting).*

Missing species and cultivated forms	Inadequate
AAA (Nangka)	Indonesia
AB	India
AAB (subgroups Maoli-Popo'ulu and Iholena)	Pacific
AAB (African plantain)	West and Central Africa
AAB (Silk, Mysore)	India
AAB (Raja)	Indonesia
Eumusa x Australimusa hybrids (AT, AAT, ABBT)	New Guinea, Indonesia, Tahiti
Fe'I	Pacific
Balbisiana (BB)	China, Indie
Callimusa species	Borneo -Indonesia
Wild species	Thailand, Indonesia
Other wild and cultivated bananas	Myanmar

Recently, the transfer to ITC of some 20 East African diploids and triploids collected in Kenya, 30 cultivars and wild specimens collected in Indonesia in 2012, and a range of Dwarf-type plantains collected in DRC is being agreed with the national collections in the respective countries.

7.1.2 The Management of the Collection at the ITC

7.1.2.1 Medium-term Storage

The ITC has approximately 1,500 *Musa* accessions, all maintained *in vitro* as tissue-culture plants under minimal growth conditions, achieved by a reduction of the ambient temperature (to 16°C) and light intensity (to 25µM.m-2.s-1, 24h/24h) in the storage room. Accessions stored under these conditions require sub-culturing every 4-22 months depending on their genotype, with a mean of 11 months across all genotypes. Accessions in medium-term storage (MTS), or active collection, are maintained under the form of proliferating shoot cultures grown on one single type of semi-solid MTS-based culture medium. It is imperative that 12-20 replicate cultures per accession are maintained in MTS in order to assure safe preservation as well as to meet requests for samples within a reasonable time span. The MTS collection forms the active collection, keeping the total diversity present in the genebank available for international distribution and use. In addition, for accessions that are not yet preserved in liquid nitrogen this collection currently also serves as base collection, assuring permanent preservation for those accessions.

The objective of the base collection (cryopreserved material) is to assure the preservation of a representative sample of the overall known and collected diversity of the genus *Musa* for an unlimited period of time. The materials are kept permanently in storage for the long-term and are not accessible for use. Materials in the base collection should only be regenerated if samples from the active collection are lost and need to be replaced.

Maintaining the material as shoot cultures in MTS is labour intensive and involves the risk of losing valuable germplasm through accidental contamination, accumulation of bacterial endophytes in cultures or the loss of the culture's morphogenetic potential. Another impediment of tissue cultures is that somaclonal variations can occur over the years, resulting in loss of genetic integrity of the genotype stored. A routine monitoring, called Field Verification (FV), has been established to ensure the viability and quality of the stored germplasm.

To secure the quality of the conserved germplasm, tissue cultures are renewed (rejuvenated) from greenhouse plants, and regenerated in the field every 10 years. This regeneration process takes place in partnership with institutes that maintain field collections and aims to validate the genetic integrity and trueness-to-type of the MTS collection using both molecular and morphological evidence.

ITC's FV activity aims to validate the genetic integrity and trueness-to-type of the MTS collection using both molecular and morphological evidence. The process allows the ITC to identify the problem accessions, remove them from distribution and replace them where possible. In order to do this, ploidy, SSR and DArT fingerprinting data (produced by the MGC and DArT Australia) are combined with morphological characterization data and photos produced by field partners (previous partners are BPI, Philippines; NARO, Uganda; CIRAD, Guadeloupe; FHIA, Honduras; CARBAP, Cameroon, and the USDA, Puerto Rico). The compiled datasets are analyzed by a panel of *Musa* taxonomists (the Taxonomic Advisory Group) in charge of validating the accessions' genetic integrity. This workflow provides quality control for the germplasm and also adds value to the content of the ITC by producing standardized characterization data that is available to users in MGIS.

During the first decade of the FV activity (2004-2014), 420 accessions have been validated out of the 855 currently available for distribution. Seventy percent of the validated accessions have been confirmed as genetically stable. However the MTS collection could be further improved by minimizing accessions that are mislabelled (6% of the 420 accessions), misclassified (6%) or off-types (2%). Many of the accessions (16%) need to be re-evaluated due to lack of robust data. The FV activity is time consuming due to the multiple datasets, partners and steps involved. Efforts are currently being made to improve the process.

The active collection serves as source material for worldwide distribution of germplasm by the ITC. Imperative for this activity is that the distributed samples are virus-free (see section Distribution of germplasm). Germplasm health management includes initial health screening, therapy if needed and full virus indexing of the MTS materials by an expert virology lab acting as a Bioversity Virus Indexing Centre (VIC) at University of Liege, Belgium or University of Queensland (UQ), Australia.

7.1.2.2 Leaf tissue collection

In 2004 the ITC also initiated establishing a tissue collection that holds lyophilized leaf samples of 883 accessions (data 2014). The lyophilized leaf collection is a utilization collection and has no conservation objective *per se*. The aim is to make a wide range of diversity readily available at low cost to molecular scientists who are using the DNA for their studies.

From a management perspective, the leaf tissue collection is for the ITC also a cost-effective way to preserve the molecular materials and representative information from each species and cultivar in the ITC, serving as a future reference for the identification of accessions in the active and base collection.

7.1.2.3 Long-term Storage

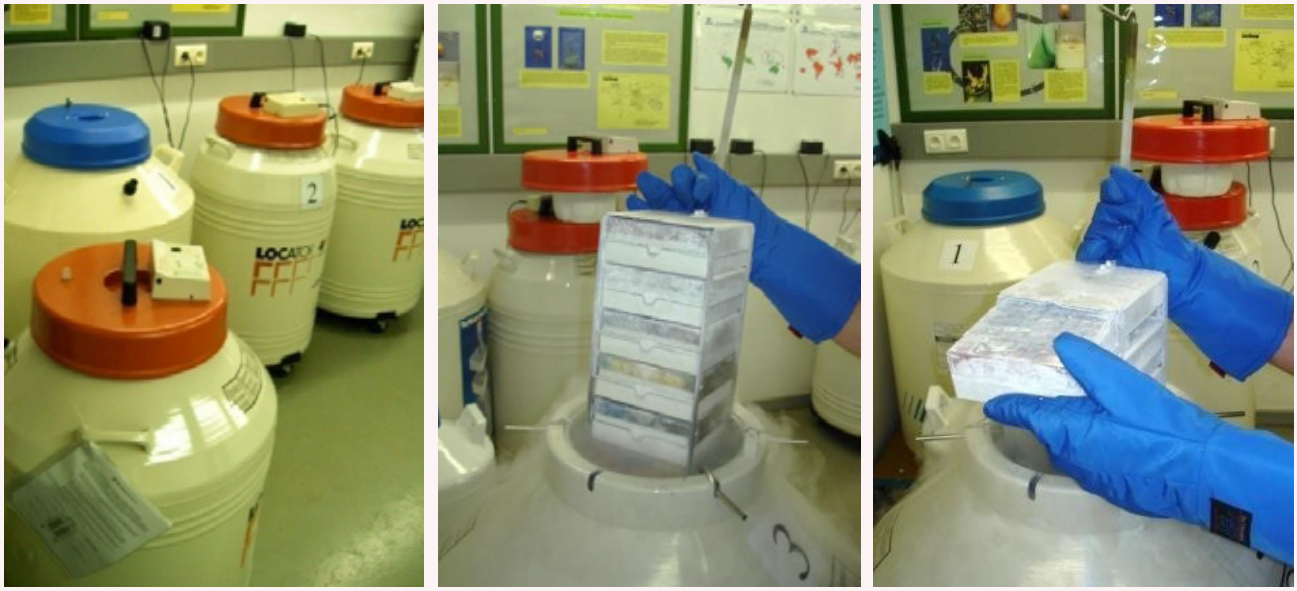
To ensure the secure and cost-effective long-term preservation of the collection, a long-term storage (LTS), or base collection, using cryopreservation has been established. Freeze-preservation research at KULeuven was carried out during the 90s and resulted in the development of three cryopreservation protocols for banana meristematic tissues that are widely applicable to the different banana groups and species. As part of the ITC *Musa* genetic resources management strategy, three replicate sets of frozen meristems are stored, each with a 95% probability that at least one plant can be regenerated.

The criteria for inclusion of accessions in the cryo-collection at ITC are that the *in vitro* material is: i) rejuvenated; ii) characterized in the field; iii) determined as healthy, and iv) free from viruses except for BSV in case of accessions containing the B-genome.

For an accession to be considered safely cryopreserved, at least three successful repetitions should be performed (i.e. 95% chance that at least one full plant can be regenerated from the material stored in liquid nitrogen) and each repetition is represented by at least 3 tubes each containing 10 meristems or meristem

clumps (i.e. at least 30 in total). For extra safety reasons, one out of the three repetitions is sent with a dry shipper to the Institut pour la recherche en développement (IRD), Montpellier, where they are stored in a 'black box' under identical conditions as the ones at the ITC.

Figure 7.1. *Cryotanks at the ITC. Photo Credit: Ines Van den Houwe.*



So far ITC has cryopreserved 938 accessions. A replicate set of cryopreserved material is held at the IRD in Montpellier, France, with 801 accessions to date. This provides an off-site, back-up as a further safety measure under the afore-mentioned 'black-box' arrangement.

Cryopreservation requires large investments to set up a facility and to process the germplasm for storage in liquid nitrogen. However, once cryopreserved, maintenance cost is very low. A study on the costs of the ITC banana genebank showed that the costs of cryostorage in perpetuity per accession are much lower than for active storage. The time period that the cumulative costs of medium-term storage and long-term storage become equal is 15 years (Garming et al. 2010). Therefore the cryopreserved base collection at ITC serves as a safety back- up that is accessed only when the accession material in medium-term storage is lost. Thanks to funds received from donors such as the World Bank, the Gatsby Charitable Foundation, the Belgian Direction Générale Coopération au développement et aide humanitaire (DGD), the CGIAR through the Genebanks CRP managed by the GCDT, 64 % of the accessions are now safely stored, making the ITC collection one of the *in vitro* collections in the world having the largest proportion of its germplasm preserved in liquid nitrogen. In a recent report entitled CGIAR Genebanks Option Paper for the Fund Council (FC) 13 (GCDT 2015), it was stipulated that if Bioversity International with the ITC banana collection reaches the performance targets listed in Table 7.3 below, by end of 2019, the full cost of the ITC core operations will be eligible for long-term funding from the GCDT.

Table 7.3. *Performance targets determining eligibility for long-term funding from the GCDT endowment.*

Indicators		Targets
1	Availability: % of the collection which is free from pathogens of quarantine risk, viable, and in sufficient quantity to be immediately available for international distribution from medium-term storage	90% of the accessions
2	Security: • Seed crops: % of the collection held in long-term storage in two locations and also in the Svalbard Global Seed Vault. • Clonal crops, % of the collection held in cryopreservation at two locations; % of the collection held in slow growth conditions *in vitro* at two locations	Seed crops: • 90% accessions in seed collections Clonal crop: • long-term target 50% accessions in cryopreservation; • intermediate target 90% accessions in *in vitro* collections
3	Data availability: % collection with minimum passport and/or characterization data available online	90% accessions in the collection
4	Quality Management System	Minimum elements of QMS/ISO are in place.

7.1.3 The Documentation of the ITC

The ITC collection is documented in two complementary database systems: the *Musa* Genebank Management System (MGBMS) and *Musa* Germplasm Information System (MGIS).

MGBMS is a system developed with/for in-house that facilitates the day-to-day management of the collection. Data related to practically all of the genebank operations, and processes are captured by the system and tracked by using accession barcodes and software that runs on mobile devices. Over the years, the system has become increasingly efficient and comprehensive, meeting the needs of the genebank staff to more effectively manage genebank data. Recently integrated new features include: the management and processing of germplasm requests, an inventory of the cryopreserved collection and leaf bank, basic characterization data and field verification data.

Accession-level information managed in this database is made publicly accessible to end users through MGIS, which holds passport and characterization data and to some extent evaluation data on the ITC accessions. MGIS also facilitates requesting germplasm from the ITC, through its on-line germplasm requesting tool. For more detail on both systems, see Chapter 9 - *Information Management*.

7.1.4 Access and Distribution of ITC Germplasm

7.1.4.1 Target users' group

The target groups directly affected by the conservation and safe exchange issues addressed here are the genebank community, the governmental and non-governmental collections, botanical gardens, conservation scientists, managers and service providers who are responsible for the sustainable conservation of *Musa* genetic resources.

Key users of the ITC are the banana research community and networks that need access to biologically well-defined material as a tool for generating new knowledge; and the banana breeders who needs sources of genes, pre-bred material and associated information to develop superior cultivars.

A survey of the direct users of the ITC and interviews with key informants was carried out as part of the 2010 ITC impact study (Garming et al. 2010). The main user groups of the ITC collection are scientists in the NARIs (40%), Agricultural Research Institutes (ARIs) and universities (30%), the CGIAR (11%), the private sector (8%), regional organizations (5%) and non-affiliated individuals (2%).

Banana farmer communities, and associated production and processing industry stakeholders and consumers ultimately benefit from improved productivity, as a result of using the conserved *Musa* genetic resources.

According to the 2010 ITC impact study (Garming et al. 2010), nearly two-thirds of germplasm requests were for the cultivated forms, mainly of local importance, and cultivars that are widely grown and demanded for trait evaluation. The most requested key traits were tolerance to biotic stress (30%), adaptation to specific local conditions and/or consumer acceptability (24%), yield characteristics (15%), tolerance to abiotic stress (13%) and pre-breeding evaluation studies (5%). Improved cultivars from breeding activities were requested for their superior yield and disease resistance characteristics and accounted for 20% of the distributed materials. Only 17% of the disseminated germplasm were wild relatives, serving as a source of potential valuable genes in breeding activities and for fundamental taxonomic/phylogenetic studies. However as mentioned above, this could be because the ITC only has 16% of wild species. Feedback on future trends anticipated an increasing demand for wild species and improved cultivars.

7.1.4.2 Legal and policy issues

When the ITPGRFA came into force in 2004 and *Musa* was included in its list of Annex 1 crops, the ITC collection also became part of the Multilateral System (MLS) of Access and Benefit-Sharing (ABS), along with all the international collections, as part of the Article 15. The exchange of germplasm occurs under the terms and conditions of the Standard Material Transfer Agreement (SMTA), for the distribution of germplasm free of charge for research, breeding or conservation purposes. The objective of the SMTA is to facilitate the process of safely exchanging and tracking use of germplasm and to encourage users to share any benefits from the use of the germplasm or resulting research products.

In some cases the acquisition of *Musa* germplasm by the ITC has been constrained by legal and policy issues mainly related to the ITPGRFA not yet being fully implemented in some countries. Acquisition may be difficult when countries are not a contracted party to the ITPGRFA, preventing unrestricted access to the materials and related information for use or safe duplication elsewhere. However, the main challenge is where certain parties have not yet put certain materials in the public domain. This could be linked to the lack of clear communication on incentives to provide materials.

7.1.4.3 Safe exchange of germplasm

The fact that the ITC is located in a country that does not produce bananas has benefits in facilitating the acquisition of germplasm from, and its distribution to, all parts of the globe without restrictive quarantine procedures.

For the distribution service, two management activities are essential: health and documentation management (described in the above section and also in Chapter 9 – *Information Management* and Chapter 10 – *Distribution and Safe Exchange of Germplasm*).

For the health management of the ITC collection, a procedure has been established that involves virus screening of all incoming plant materials, the application of virus therapy, if needed, and subsequently the full virus indexing of the accession material stored in the collection. This work is carried out by contracted specialized laboratories following the MusaNet publication *Technical Guidelines for Safe Movement of Musa Germplasm (3rd edition)* (Thomas 2015). The guidelines, describe technical procedures that minimize the risk of pest introductions due to the movement of germplasm for research, crop improvement, plant breeding, exploration or conservation. The guidelines were revised and published and are further described in Chapter 10 – *Distribution and Safe Exchange of Germplasm*.

The ITC collection is widely recognized as the safest source of *Musa* germplasm. Therefore, the international distribution of germplasm is mainly from the ITC because of its comparative advantage that tissue culture samples of the most comprehensive set of *Musa* diversity can be obtained from a single source and the health status is guaranteed. However, only small sample-sizes of five plants per requested accession can be provided.

In the Asia-Pacific area, national repository and dissemination centres have been established in 13 countries to support of the distribution activity of the ITC. The Secretariat for the Pacific Community (SPC), in Fiji, also acts as a regional distribution centre for the Pacific region. Even so, this additional capacity is insufficient to meet all users' needs. To some extent this is addressed by referring users to local *in vitro* labs where the material can be multiplied, however the capacity is also limited.

Few other collections are a reliable source of disease-free material and in many cases germplasm dissemination poses risks of introducing viruses or other diseases, and can be constrained by other quality issues.

However, the major issue impeding germplasm distribution from the ITC is related to BSV which can be integrated in the B-genome of banana, and can be activated to cause episomal viral infections under some circumstances. *In vitro* conditions are a factor that increases the probability of the virus to be expressed. The episomal form of the virus may cause damage in terms of yield losses when infected plants are cultivated in the field. Presently, no therapeutic techniques are available to clean tissue cultures from the integrated form. However for genotypes with exclusively the A-genome, effective remediation is possible since viral sequences related to BSV, although integrated in the A genome, are fragmented and unable to liberate the episomal form of the virus.

In August 2014, a workshop at the ISHS/ProMusa Symposium addressed these concerns, and a task force was formed to discuss possible solutions to the problem of BSV limiting distribution. In May 2015, the BSV Task Force developed a position paper on strategies to enable the distribution of BSV-infected *Musa* germplasm that was evaluated by the *Musa* community via MusaNet and ProMusa and posted on the MusaNet web site (MusaNet 2015b). The implementation of this strategy, planned for late 2016, will allow users to have access to 90% of the material maintained at the ITC, which is one of the performance targets listed in Table 7.3. For more details see Chapter 10 - *Distribution and Safe Exchange of Germplasm*.

The ITC's accessions are frequently requested by external users for *Musa* diversity. Since its foundation in 1985, the ITC has distributed more than 17,000 accessions to more than 200 users based in 103 different countries. Looking at the yearly use of the collection the analysis of the number of distributed accessions revealed an average utilization ratio of 22% (see Figures 7.2 and 7.3). Considering only the fraction of the collection that is accessible for distribution (virus indexed negative) samples of 35% of the available accessions are distributed.

Figure 7.2. *Yearly number of accessions distributed since 1985.*

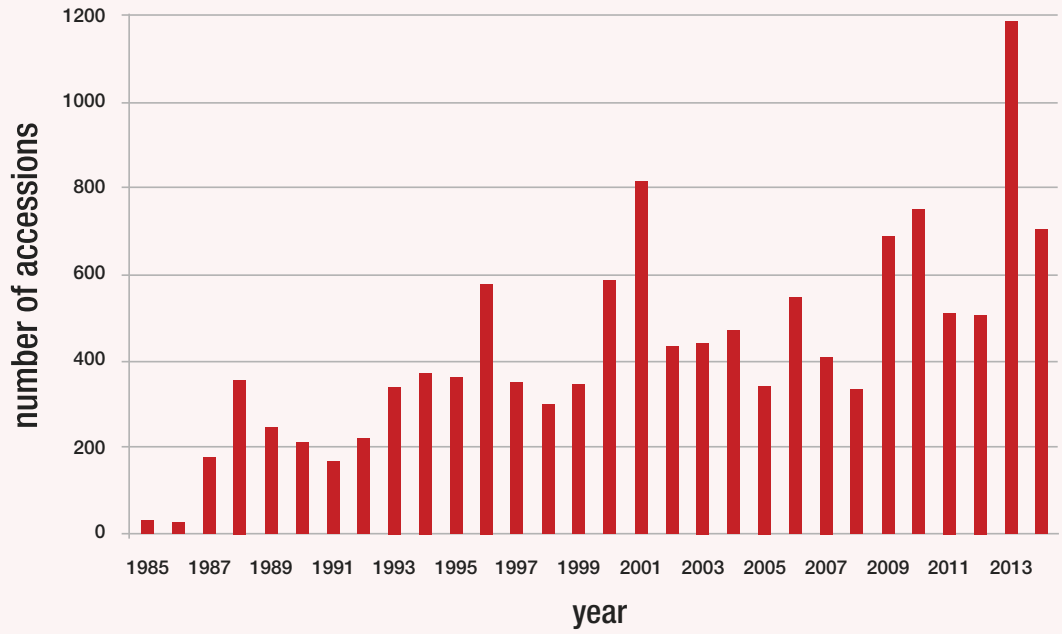
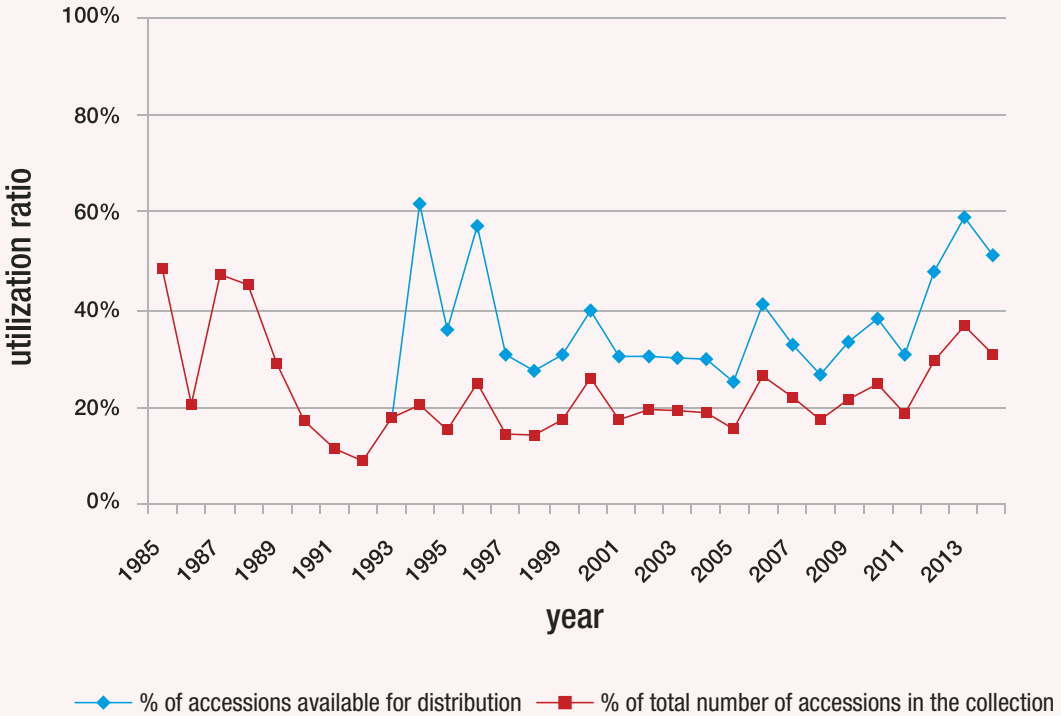


Figure 7.3. *Yearly utilization ratio[1] of the collection.*



1 The utilization ratio is calculated as the number of distributed accessions per year divided by the number of accessions present in the collection (red line) or the number of accessions available for distribution (blue line) in that year. Before 1993, when no virus indexing techniques were available, all accessions in the collection were available for distribution.

There has been a clear boost in distribution in 2013 for the following reasons:

- The publication in *Nature* in July 2012 of the 1st article on the whole *Musa* genome sequence (D'hont *et al*. 2012).
- The introduction of the *Musa* Online Ordering System (MOOS), allowing users to order germplasm from ITC more easily.
- Major proportion of the collection available in the form of lyophilized leaves.

The distribution of guaranteed healthy germplasm has enabled and facilitated a large body of research and played an important role in the implementation of banana-related development projects.

The most important areas where impacts from increased distribution have been created are:

- Supply of a broad genetic diversity to the breeding of superior banana germplasm.
- Fundamental research carried out with materials from the ITC .
- Research on resistance/tolerance to economically important banana pests and diseases.
- Dissemination of superior germplasm to small-scale farmers with expected positive effects on their productivity.

SECTION 7.2 THE ITC GLOBAL *MUSA* COLLECTION - WHERE WE WANT TO GO

7.2.1 A global core collection of *Musa* biodiversity

As stated elsewhere, the five objectives of the ITC are: i) provide long-term and sustainable conservation of *Musa* genetic resources; ii) maintain a source of genetic diversity and related information in the public domain; iii) contribute to understanding *Musa* diversity through characterization; iv) provide a service for the safe movement of germplasm and related information, and v) develop and transfer *ex situ* conservation technologies. As mentioned above, all material in the ITC is preserved *in vitro* with 64% of the accessions backed up in liquid nitrogen, and although the ITC maintains strong links with the field collections, the sustainable field conservation component needs to be formally developed, particularly as some accessions are permanently represented within *in vitro* collections only. Linked to the first objective, a 'core' collection of accessions, representing the total *Musa* diversity, will be identified and maintained in both field genebanks and at the ITC. Establishing this global core of representative *Musa* diversity across several designated sites for conservation in perpetuity will be achieved in the long-term. The core collection will also be a useful tool providing evidence of gaps in the collection and thus contribute to the improvement of conservation. Through targeted collecting and duplication of unique accessions from national collections, the collection will be further expanded to increase its coverage of *Musa* diversity.

7.2.2 The entire ITC collection cryopreserved ensuring safe long term preservation

A constraint for the ITC is the high initial cost of applying cryopreservation. Costing studies have confirmed that cryo-conserving *Musa* germplasm is initially expensive, largely due to high labour requirements to process the plant material in liquid nitrogen. Although the ITC genebank has assured financial support for the long-term through the GCDT, 40 % of the collection has not been safely backed-up in liquid nitrogen with funding being the limiting factor. Recognizing the important role of the ITC in the global conservation effort for *Musa*, it is critically important that cryopreservation of the entire collection is achieved in the foreseeable future.

7.2.3 Increasing the availability of ITC materials for distribution

Viruses, especially BSV, are the major constraint for distributing *in vitro* germplasm internationally. Plant health testing methods are evolving rapidly, as new diseases (virus and phytoplasma) emerge. Up-to-date

knowledge and technologies also need to be transferred to regional laboratories to increase awareness and maximize the health status of collections and facilitate safe exchange of germplasm at the regional and national level. BSV is a major impediment to the international transfer of valuable germplasm from the ITC. Today 25% of the ITC germplasm cannot be moved because of BSV. The GCDT commissioned a review of the ITC in 2013, and recognized the need for an informed and inclusive discussion on the relative risks and advantages of distributing this material, and seek a consensus among stakeholders (for more information, see the position paper prepared by the BSV Task force and published on www.musanet.org).

7.2.4 Ensuring the genetic integrity of the ITC collection

As the first round of the Field Verification activity is nearly complete, a review of the procedures, as recommended by the GCDT, will be undertaken. In order to improve the accuracy and efficiency of the activity, decisions need to be taken on the optimal length of time accessions remain in medium term storage before being field verified, which involves a close review of the first round results, especially in terms of possible mutation due to somaclonal variation. Also required is a review of the overall process, including field partners and TAG expert members that contribute to the exercise. Streamlining documentation of FV is also a priority, and one effort currently underway is to optimize the input process through use of a MGIS dashboard where TAG members can easily access data and photos.

7.2.5 *Musa* seed conservation

The greater part of genetic diversity in *Musa* lies within its wild species, the majority of which are not kept safely in *ex situ* collections. Users of the ITC indicated that there is an interest in this wider diversity which is still under-represented in the collection and not often accessible from other collections. These wild species could serve as sources of alleles for specific traits (e.g. BLS or Fusarium wilt tropical race 4 resistance; drought stress tolerance; superior nutritional value) meeting the needs for current or future banana improvement. In order to capture and secure the diversity present within wild populations and to broaden its accessibility for breeding, habitat restoration, and scientific research, seed conservation needs to be explored as a complementary conservation approach.

Banana seeds could be distributed using an SMTA and link to the Bioversity ITC collection/database through the *Musa* On-line Ordering System, MOOS. Requests for seeds will go through MGIS-MOOS.

7.2.6 Increased Access and targeted use of the ITC collection

A constraint to using *Musa* genetic resources in collections, including that of the ITC, is that accession documentation is incomplete. Efforts are underway through the FV activity, to characterize the ITC accessions systematically using morpho-taxonomic descriptors complemented by molecular analysis.

In addition, it was recommended that ITC take a more proactive approach to collect evaluation data. The collected data will not only add value to the diversity conserved but lead to an increased and more efficient use of the collection based on better knowledge about specific traits of accessions. A better documentation of the collection will also allow the identification of several smaller collection 'subsets' expressing certain desirable traits for study, which will be particularly beneficial to increase the efficiency of germplasm use in evaluation and breeding. Such subsets will assist the ITC in providing user groups with a manageable, standardized set of accessions to undertake particular studies as well as to generate standardized information. Over the years, three 'subsets' have been identified and developed from the ITC collection: i) the Taxonomic Reference Collection consisting of 34 accessions (see the TRC project in Chapter 4 – *Taxonomy* and Chapter 5 – *Characterization*); ii) the International *Musa* testing Programme (IMTP) 'reference' sets, established for evaluating disease resistance/tolerance in different agro-ecological environments; and iii) a 'mini-core' of 52 accessions, developed in the framework of the Generation Challenge Programme (GCP) for genomic studies. The diversity in the collection will be documented in MGIS, offering users access to more complete characterization data and trait evaluation data.

SECTION 7.3 THE GLOBAL *MUSA* COLLECTION - HOW WE WILL GET THERE

7.3.1 Establishing a global core collection of *Musa* diversity

Identification and setting up of a global core collection of *Musa* diversity in several designated sites for *in-perpetuity* conservation will involve a step-wise process of identifying the materials to enter the core. In the interests of long-term conservation, and as a result of collaborative characterization efforts initiated by the TRC project, priority lists of core accessions will be drafted for specific national, regional and global collections, and candidate accessions for the global core collection will be identified.

The commitment of several partner collections to participate in this global initiative will be required. The effective conservation of maximum *Musa* diversity will be assured in a permanent manner through a functional network of collections that are actively contributing and benefitting from shared standards, technical capacity, and germplasm and information exchange. Partnership agreements will need to be established with regional and national field collections, to share complementary responsibility for preserving global core accessions. The ITC will hold the responsibility to duplicate the global core accessions *in vitro* and in cryopreservation. A safety back-up will be maintained off-site.

The ITC collection will also be strategically expanded by filling the gaps in conserved diversity, especially with respect to wild species through targeted collecting. Following a comprehensive gap assessment, a gap-filling strategy will be developed for collecting new diversity and sharing missing diversity from existing collections in the ITC collection. The gap analysis and collecting under the GCDT-coordinated Crop Wild Relatives Project will contribute significantly to this task.

7.3.2 Cryopreservation of the whole ITC collection

To meet the objective ensuring the safe long-term conservation of the global collection, about 500 more accessions need to be processed. Samples from the *in vitro* collection are actually being cryopreserved at a rate of 40 accessions per year. The Genebanks CRP contributes funding for the cryopreservation of 25 accessions per year and in addition 120 backlog accessions will be processed by the end of 2016 within the framework of Genebanks CRP-funded project. To ensure the safe long-term preservation of the entire ITC collection, still more than 300 accessions will need to be cryopreserved from 2017 to 2020 and backing up the cryopreserved collection at an off-site location (IRD, France) will be pursued.

7.3.3 Increased capacity to develop healthy germplasm for conservation and use

To comply with latest standards, the 1996 *Technical Guidelines for Safe Movement of Germplasm for Musa* have recently been revised (Thomas 2015). This was a MusaNet initiative, conceived at the launch meeting in 2011, and was carried out in partnership with plant health laboratories and specialist pathologists in order to increase ownership and acceptance of these standards among stakeholders.

In order to minimize the risk of transferring virus-infected material to ITC, it is also important that the field collections have regional access to appropriate indexing expertise. Where necessary, training in indexing methodologies should be given. An accreditation system for indexing laboratories would allow more confidence in the regional movement of germplasm.

Health testing is also a costly and lengthy procedure for the ITC and other collections. Options need to be explored to enhance the cost-efficiency of the indexing process.

The moratorium on the distribution of BSV infected accessions is being reconsidered. In this respect, a taskforce of banana virologists and stakeholders was set up to advise the ITC on the management of virus infected materials. A strategy to minimize the risks associated with the distribution of B-genome germplasm containing banana streak virus, while ensuring that the recipient country and users are fully aware of the

potential issues, was developed. Its acceptance by the banana community opens up the possibility to release 300 B-genome accessions for distribution. An intensification of the sanitation activity for other viruses than BSV, present in these accessions, will result in an increase of the percentage of the ITC collection availability for distribution from 60% to 90% in the coming years.

7.3.4 Increased access and targeted use of the ITC collection

Optimization of the use of the collection will be achieved if substantial investments in characterization (morphological and molecular) and evaluation are made. The ITC field verification protocol will be reviewed and links with partners will be reinforced (in collaboration with USDA, Puerto Rico) to collect standard characterization data. The entire ITC collection will be characterised by SSR by end of 2016. The analysis of the results will help detect duplicates in the collection and identify genetic clusters.

The formation of several smaller collection 'subsets' expressing certain desirable traits for study, will be beneficial to increase efficiency of germplasm evaluation and enhance knowledge of the diversity the collection.

Access to germplasm and associated information will be increased through better links between ITC and MGIS, providing regular updates on availability of new germplasm or new information on ITC accessions. The system should also integrate a feedback mechanism (e.g. conducting of user surveys) for information on distribution of ITC germplasm and monitoring the use, including the collection of evaluation data. This will allow ITC to create proactively new subsets with traits of interest for potential user groups.

Use of the collection will be promoted through the new MGIS website, offering user-friendly functions for online ordering of accessions and trait specific accession subsets.

7.3.5 Preserving the wider *Musa* wild diversity through seeds

Exploring the feasibility of seed conservation for preserving the wider wild diversity as complementary approach will be an important activity. It has been demonstrated that seed cryopreservation combined with embryo rescue works for a number of species, and offers potential as conservation method, but it needs to be tested more widely. Investigations should also focus on aspects of the seed physiology to improve the understanding of germination requirements, seed storage behaviour, and the genetic structure of populations of wild *Musa* species in order to develop an appropriate sampling strategy for seed banking.

Through the RTB-CRP, an international collaborative programme, particularly involving strategic partners in Asia and the Pacific, should be set up to investigate the feasibility of seed banking. Currently, a study on population genetics of banana is being undertaken by Bioversity, the Millennium Seed Bank at Kew Gardens, UK, and Meise Botanical Gardens, Belgium.

A summary of the proposed actions concerning the ITC collection is set out below in Table 7.4.

Table 7.4. *Summary of the key objectives and proposed actions for the ITC collection.*

Specific objectives	Actions
Identification and setting up of a global core collection of *Musa* biodiversity in several designated sites for in perpetuity conservation	• Developing priority lists of accessions (core sets) for specific collections (national, regional, global) for long-term conservation • Identification of candidate accessions to enter the global core collection • Establish partnership agreements with regional and national field collections for complementary responsibility sharing to preserve global core accessions • Global core accessions duplicated *in vitro* and in cryopreservation at the ITC. Safety back up of the ITC cryopreserved collection • Targeted collecting and duplication of unique accessions from national collections, increasing the coverage of the known *Musa* diversity in the ITC collection

Specific objectives	Actions
Increase access and targeted use of the ITC collection	• Developing priority lists of accessions (core sets) for specific collections (national, regional, global) for long-term conservation • Identification of candidate accessions to enter the global core collection • Establish partnership agreements with regional and national field collections for complementary responsibility sharing to preserve global core accessions • Global core accessions duplicated in vitro and in cryopreservation at the ITC. Safety back up of the ITC cryopreserved collection • Targeted collecting and duplication of unique accessions from national collections, increasing the coverage of the known *Musa* diversity in the ITC collection
Increase access and targeted use of the ITC collection	• Link between ITC and MGIS database to create feedback mechanism for information on the ITC collection germplasm exchange and use • Promote the use of the on-line ordering tool running on MGIS for the global ITC collection. There is a new MGIS website with more user friendly functions for ordering accessions • Field verification, morpho-taxonomic characterization , flow cytometric ploidy determination and genotyping of the ITC collection to ensure the genetic integrity and improve the documentation status of conserved accessions • Identification of accession 'subsets' expressing certain desirable traits of interest for potential user groups • ITC to proactively distribute germplasm to collections with specific interests for specific regions, and indicate these as subsets in MGIS
Increased awareness of the need for high health status germplasm	• Update the Technical Guidelines for the Safe Movement of *Musa* Germplasm to incorporate newly discovered viruses and the latest indexing methods. Update disease Factsheets
Improve the efficiency of virus indexing protocols	• Review current indexing protocols to highlight deficiencies and inefficiencies
Seek a consensus on the risks of distribution of integrated, activable BSV in germplasm	• Bioversity to develop a position on the movement of germplasm with integrated, activatable BSV based on the relative risks and advantages to the recipient country, and the responsibility of the germplasm supplier. A position paper was developed by the BSV task force and is posted on the MusaNet web site.
Secure the long-term conservation of the entire ITC collection	• Cryopreserve the entire ITC collection • Safety-back up of the entire cryopreserved collection at off-site location (IRD, France)
Expand long term conservation capabilities by seed banking	• Explore the feasibility of seed conservation for preserving the wider wild diversity as complementary approach

CHAPTER 8.
IN SITU AND ON-FARM CONSERVATION

SECTION 8.1 *IN SITU* AND ON-FARM CONSERVATION - WHERE WE ARE NOW

The conservation of *Musa* genetic diversity is carried out by the following three complementary methods:

- *In situ* conservation, i.e. in the wild natural habitats where specific species evolved and continue to do so
- On-farm conservation, i.e. in farmers' fields where continuous cultivation, adaptation and improvement of cultivars is often carried out by small-scale farmers cultivating traditional local cultivars (including home gardens)
- *Ex situ* conservation, in a field research station, *in vitro* genebank (medium-term) and/or in cryopreservation (long-term).

The conservation in *ex situ* collections is to ensure long-term safeguard and accessibility of representative samples of the entire *Musa* genetic diversity, whether threatened today or to be in the future and this is discussed in Chapter 6 – *Musa collections around the world*. This chapter focuses on the complementary methods of conservation: *in situ* and on farm.

The Convention on Biological Diversity (CBD) defines *in situ* conservation as "the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties" (CBD 1992). On-farm conservation is therefore often regarded as a form of *in situ* conservation but in many cases, the reasons why farmers continue to grow traditional cultivars may have little to do with conservation and much more to do with traditions and preferences, risk avoidance, local adaptation, niche market opportunities or simply the lack of better alternatives (FAO 2010). On-farm conservation therefore provides new challenges in that there is need to understand the social, economic and cultural aspects of the traditional farming systems, seed supply system as well as farmer-based breeding and experimentation (Hodgkin et al. 1993). There are two distinct activities that are considered to be on-farm conservation. One is when the focus is on the conservation of genetic diversity of the crop, within a particular farming system, and the other is when the focus is on the conservation of the traditional farming system, irrespective of what happens to the genetic diversity of landraces within the farming system (Altieri and Merrick 1987).

In this document, *in situ* conservation of plant genetic resources for food and agriculture (PGRFA) will mainly refer to the conservation of wild species in protected areas and in habitat management outside of protected areas. On-farm conservation will refer to the conservation of cultivars in production systems. The CBD recognized the continued maintenance of traditional crop cultivars on farms by different farming communities as an essential component for sustainable development. Furthermore, the Global Plan of Action (GPA) has recommended the compilation of inventories of all plant genetic resources for food and agriculture; increased support for on farm management and improvement of these resources; and assistance to farmers in disaster situations to restore agricultural systems and promote on-farm conservation of crop landraces, wild crop relatives and wild plants for food (FAO 1996).

The great advantage of *in situ* and on-farm conservation is that the evolutionary processes of the wild species and traditional cultivars are maintained in a dynamic way. The key variables in this dynamic process are the following (Brown 2000):

- Genetic diversity within populations, which is the essential raw material for evolution

- Breeding system variation (such as changes in the out-crossing rate)
- Variation in resistance in space and time, related to pest pressure and diversity, and
- The dynamics of seed systems, persistence and migration

The wild species and traditional cultivars thus need to be conserved if they are to be effectively utilized in breeding programmes. While wild taxa can be traced and conserved in forests, their primary centres of diversity, landraces or traditional cultivars very often can be traced to farms, which act as secondary centres of diversity. These on-farm crops contain less genetic diversity than their wild ancestors, but they contain more variation within the field populations than modern cultivars, (Pickersgill 1994) such as Cavendish.

Relative to *ex situ*, the conservation of *Musa* genetic resources *in situ* and on farm is less studied, as they are often influenced by complex social, political and biological factors. In addition, there is a threat to the continuity of habitat for *Musa* growing in the wild.

In 2013 and 2014, Bioversity carried out a literature review of past and current *Musa in situ* and on-farm conservation projects. The report, titled "*Musa spp. Landraces and Wild Relatives: Towards a framework for On-farm Management* and *In Situ Conservation Strategies*" (Bioversity International 2014) was the first step toward a better understanding of the situation *in situ* and on farm. At the time, Bioversity requested ProMusa members to provide information on known past or current on-farm and *in situ* conservation activities as well as on people and organizations who may have knowledge and expertise in this area. The authors stressed that the most powerful reason for the increasing awareness of *in situ* and on-farm conservation is their potential use in crop improvement and that wild species maintained *in situ* and traditional cultivars maintained on farm are potential gene donors for breeding programmes. The report also suggested that expected changes in climate will require modifications in production systems and adaptation of cultivars to extreme and fluctuating environmental conditions and it is likely that most of the desired traits required for breeding are already contained in wild species and traditional cultivars. Furthermore, diversity conservation in agricultural landscapes augments the resilience of these areas and therefore, increases possibility of adaptation to climate changes.

8.1.1 *In situ* conservation

Wild *Musa* species naturally occur in South-East Asia, including eastern India, Papua New Guinea and the northeastern Australia. The commonly accepted boundaries for their distribution are presented in Figure 1.2 - *Distribution of the four sections of the genus Musa*. The wild *Musa* taxa represent up to 70 different species, subspecies and varieties (list from Häkkinen 2013), some of the species, such as *M. acuminata*, being subdivided into a number of subspecies. However, quite few specimens of these taxa are conserved in *ex situ* collections where, for example, 12% of the accessions in the field collections are of wild taxa, and 15% in *in vitro* collections (see Table 6.2 - *Number of accessions of different types of germplasm in the field* and *in vitro collections*).

The under-representation of wild *Musa* species in *ex situ* collections is also coupled with the under-representation conserved in terms of number of different accessions per taxon. For example, some sub-species of *M. acuminata* are only represented by a single clone, such as *microcarpa*, *truncata* and *sumatrana*. Of the 15 "botanical varieties" of *M. acuminata* in Indonesia described by Nasution (1991), several correspond to more generally recognized sub-species, and many are not represented in the ITC. This aspect highlights the fact that the within-species component of the diversity is very often neglected in *ex situ* collections probably due to insufficient sampling or poor sampling strategy during collecting. In addition, wild *Musa* species are indeed more difficult to conserve *ex situ* since seeds conservation techniques are still under development (see Chapter 6). However, this pattern can also be explained by

additional factors. In view of an increasing reluctance to provide access to wild resources from many countries due to the lack of or unclear policy and legal frameworks for these species, *in situ* conservation within countries has become a priority to ensure their safe conservation.

In situ conservation involves the protection and management of the areas, ecosystems and habitats in which the plants have developed their distinctive characteristics, and is facilitated through legislative and policy measures as well as the use of incentives. *In situ* conservation has clear potential for species conservation, as it supports the processes that allow the species to adapt and evolve. The potential of wild *Musa* species for breeding purposes and for the development of improved cultivars has increased the awareness of the need to protect *Musa* crop wild relatives (CWR). Very little is known about wild *Musa* species being conserved in protected areas and for those of *Musa* CWR that were reported growing inside such areas, no specific management plans exist and populations are not actively monitored. Most wild *Musa* genetic resources are located outside protected areas, in ecosystems such as riversides, mountains and forests, and many of these species survive in human-made habitats, such as on roadsides and on the edges of cleared land. Several wild species are distributed in restricted areas and are suffering genetic erosion due to habitat destruction, stochastic events and possible impacts of climate change (Maxted and Kell 2009). As planters in Indonesia and Malaysia say, banana is only the worst weed in new plantations for the first three years, after which it's no longer much of a problem (i.e. the seedbank and corresponding genepool are gone). No *in situ* conservation projects for wild *Musa* could be found in the 2014 Bioversity literature review (Bioversity International 2014). Based on the little data available, the general *in-situ* conservation status of wild *Musa* is thus worrying and needs urgent attention.

8.1.2 On-farm conservation

A significant amount of *Musa* diversity continues to be maintained in farmers' fields. Many farmers are already practicing *de facto* on-farm conservation through the continued cultivation of landraces or traditional cultivars of bananas and plantains. Such cultivars will be conserved as long as they have productive potential or cultural interest and continue to be cultivated by farmers. It is, in effect, conservation through use. The conservation of such traditional cultivars by farmers differs in some important aspects from the *in situ* conservation of wild material. In the case of *Musa*, cultivars are most often sterile and do not reproduce sexually, so there is no exchange of genetic material between individuals or within populations. Whereas the conservation of wild species is focused on conserving diversity at the population level, conservation of cultivars focuses on individual genotypes in which some changes may occur as a result of clonal diversification mechanisms (e.g. accumulation of mutations).

Traditional cultivars have generally been selected by humans to suit the environment in which they are cultivated and the particular needs of the grower. They are the result of domestication, followed by constant diversification through farmer selection. Traditional cultivars do not however remain static; they continue to evolve and develop. In the case of *Musa*, new, beneficial mutations occur from time to time. These are recognized by the farmer who consciously selects such individuals and then continues to maintain them.

In India, an impressive diversity of cultivars with specific uses has been maintained in small rural areas. As reported during the TAG meeting in 2006, more than 90% of small villages are estimated to have stopped growing these traditional cultivars and very few have been commercialized and cultivated on a larger scale. Some species and cultivars, including those recently discovered, can no longer be found in their original collecting sites, for instance in the Andaman and Nicobar Islands. The Fe'I, Maoli-Popo'ulu and Iholena subgroups are similarly poorly collected and conserved.

Through the 2014 Bioversity literature review, only four on-farm management projects for *Musa* landraces were identified, and these were implemented in Uganda, Tanzania, India and Ecuador (see Table 8.1). Other projects certainly exist but were difficult to identify due to lack of published information on them.

Home gardens are reservoirs of *Musa* landrace diversity. There are many projects with the main objective of improving farmers´ livelihoods, through a focus on banana cropping systems and on helping farmers overcome the constraints that are limiting their production. Although the aim of these projects is not the conservation of diversity, they are implemented in areas with high diversity, and are likely to be affecting it.

Table 8.1. *A summary of the four on-farm management projects to conserve* Musa *diversity inventoried through the 2014 Bioversity literature review.*

Project Name, Institute and Date	Main objectives	Countries	Species or Cultivars	Results
Utilization of banana (*Musa* spp.) based biodiversity to improve livelihoods in East Africa, INIBAP, NARO and ARI, 1999-2006	Develop management strategies that would enhance conservation of *Musa* genetic diversity at farm level. Phase I - to assess the diversity on farm and identify the constraints faced by farmers. Phase II - to improve livelihoods of farmers and encourage the conservation of *Musa* diversity by developing uses and finding markets for this diversity.	Uganda and Tanzania	Implemented regardless of cultivars grown, but the main cultivars grown in the area were East African Highland bananas (EAHB), AAA-genotype.	1. Banana diversity in the region was characterized and mapped. 2. Improved agronomic practices to arrest genetic erosion were disseminated. 2. The associated diversity in banana-based systems has been characterized. 3. Indigenous knowledge was identified and validated, 4. Associations were strengthened. Teaching centres were organized and demonstrations of good practices spread. 5. Banana diversity at the benchmark sites was increased. 6. Five community genebanks are operational. 7. New banana-based products were marketed. 8. Information products were disseminated.
Conservation and use of crop genetic diversity to control pests and diseases in support of sustainable agriculture" implemented by Bioversity and the Plant Genetic Resources Center of NARO and funded by UNEP/GEF. (2004-2015)	Use crop genetic diversity to enable farmers to reduce pest and disease pressure and enhance sustainable agricultural production.	China, Ecuador, Morocco and Uganda	12 cultivars in Ecuador (AA, AAA, and AAB) 30 cultivars in Uganda.	Project still ongoing

Project Name, Institute and Date	Main objectives	Countries	Species or Cultivars	Results
Conservation of Hill Bananas of Palmi Hills, NRCB, TNAU, Tamil Nadu Banana Growers' Federation and Tamil Nadu State Horticultural Dept., 2002-2006	Reintroduction and rejuvenation of the hill bananas to improve the livelihood and nutritional security of the banana farmers and consumers.	Tamil Nadu (India)	Hill bananas, Virupakshi and Sirumali cultivars (AAB genome - Pome group)	1. Rejuvenation of the plants 2. Increase in cultivated area 3. Increase in production
Rejuvenation, *in situ* conservation and cultivation of nearly extinct banana landraces of Kolli Hills, Tamil Nadu, NRCB, Department of Horticulture and M S Swaminathan Research Foundation, 2011-ongoing	Rejuvenation and conservation of traditional banana cultivars.	Tamil Nadu (India)	Two cultivars of fragrant bananas: Karuvazhai/ Manoranjithan (AAA genome) and Numaran/ Ladan cultivars (AAB genome - Pome group)	Project still ongoing

SECTION 8.2 *IN SITU* AND ON-FARM CONSERVATION - WHERE WE WANT TO GO

On-farm conservation values have already been identified as an integral component of the culture of most banana growing communities. Additionally, some conservation initiatives include other forms of biodiversity in *Musa*-based systems, which contribute importantly to soil and human health. This situation calls for a holistic approach that requires a number of players, in order to implement *in situ* and on-farm conservation.

The purpose of this chapter is to propose an action plan to guide conservation initiatives and to provide a way to sustainability once implementation is to be achieved. The first requirement for success is the participation of the widest possible range of actors who should define issues and identify possible actions, including beneficiaries and implementers.

The action plan (*in situ* and on-farm methodology) should include the following items:

- Site selection
- Means of monitoring the strategy's implementation
- Main processes and activities in implementing the strategy
- Emphasizing the complementary aspects of *ex situ* and *in situ*
- Measuring and assessing diversity (understand the status of diversity in selected sites)
- Monitoring diversity
- Locating diversity and custodians
- Community sensitization
- Integration of on *in situ* and on farm methodologies into national programmes
- Link with national/regional seed system
- Different roles of the different stakeholders indicating an effective coordination of activities
- Policy advice and recommendations that are necessary and sufficient to solve the problems identified as well sustainability of the conservation process

To ensure the sustainability of *in situ* and on-farm conservation, it is critical that effective utilization remains part of the long-term strategy to strengthen diversity conservation objectives. This statement is true considering on farm conservation in smallholder systems where resources (land, funds) are very limiting, farmers will have to make critical decisions based on basic needs. During the on farm studies (Karamura et al. 2004) it was observed that farmers selected and conserved those varieties that met their food income and cultural needs to fit them in the 0.5-3.0 acres they owned. In addition, the overall knowledge concerning the different conservation methodologies and their practical use should be integrated into national conservation programmes.

8.2.1 *In situ* conservation

There is a lack of appropriate and complete information about *Musa* diversity that would allow the development accurate and effective *in situ* conservation initiatives. For example, *ex situ* collection data is used to build distribution and diversity maps, but these data only demonstrate the presence of the species at a given place and at a specific time, and do not give any information about the conservation status.

Collecting and characterizing new genetic resources should be done to prevent resources from being lost from their natural environments (see Sections 6.1.10 and 6.2.2.2 on gaps in collections). Indigenous knowledge of wild species is important to maintain as it provides valuable perspectives on the potential use and value of wild species. However, TAG 2006 suggested that many *Musa* species that are being used may be loosely qualified as “wild” as they may have been selected and domesticated at some extent for other traits than the fruit (e.g. for ornamental purposes). There is therefore a particular objective in identifying and focusing specific conservation efforts on purely wild populations to represent genetic diversity in the evolving natural system.

In practice, strategic alliances should be developed with various initiatives such as national forest reserves, wildlife refuges and private reserves. Partnership between government agencies, scientific research institutions, NGOs, farmer communities and possibly the private sector should be fostered. Collaboration with botanic gardens is also particularly important to tap into their taxonomic and conservation expertise of wild species. For example, Forest Research Institute in Malaysia is building

A collection of wild banana species and collections is also held in Chinese and Hawaiian botanic gardens. Botanical Gardens Conservation International (BGCI), an international organization that supports the development and implementation of the Global Strategy for Plant Conservation (GSPC) at the global, regional, national and local levels[2], also links very closely to the International Union for the Conservation of Nature (IUCN) and their development of a red list of threatened species.

NRCB India took the lead in providing details of threatened *Musa* species for the Indian Red Data Book. As recommended by TAG 2006, a global red list should be produced to identify priorities and further monitor genetic erosion of wild *Musa* species. It will require a global effort to identify and locate wild *Musa* resources. Such an effort could be achieved through a crowd-sourcing approach[3] that would help gather the required information from local people. It will also demand that those collecting germplasm ensure that appropriate levels of details are gathered on geographical locations (using a global positioning system), environments, habitats and population status information.

8.2.2 On-farm conservation

Although numerous activities and projects have been implemented within banana cropping systems, the focus has been on introduction of improved cultivars, improvement of banana production and control of pests and diseases. The attention paid to the management of diversity has been limited.

In reality, *Musa* diversity is still conserved in small-scale and poor farmers’ fields. However, there is little

2 http://www.bgci.org/files/Plants2020/popular_guide/englishguide.pdf

3 Telebotanica is an appropriate example of the crowd sourcing approach (see http://www.tela-botanica.org)

information about which landraces are grown by farmers, which farmers are growing these landraces, and why and what are the main constraints they face to keep growing them.

Efforts to conserve diversity on farm need also to take into account the dynamic system in which banana diversity is being maintained from production to consumption. Farmers make the ultimate decisions about the conservation and use of particular cultivars in their community. It is therefore critical to understand the socio-economic determinants that influence their decisions. Traditional cultivars often have lower yield but can also be important sources of resistance to pests and diseases. On-farm conservation can be strengthened through activities in the farming community, such as participatory cultivar selection, breeding and farmer field schools. Therefore, a major *in situ* and on-farm component of the Global Strategy should focus on the generation and management of pertinent socio-economic and biological information on this precious resource.

To increase efficiency, conventional conservation efforts need to be coordinated with national seed systems. The experience from the existing on-farm conservation initiatives provides important learning that should be shared among the wider community. One of the major concerns of on-farm conservation projects is their sustainability. Conservation has to be linked to income generation (Sharrock and Frison 2004) and organized with communities that already have strong, diverse production systems. Experiences and expertise in this area should be shared with the wider *Musa* community.

SECTION 8.3 *IN SITU* AND ON-FARM CONSERVATION - HOW WE WILL GET THERE

8.3.1 *In situ* conservation of *Musa* Crop Wild Relatives - CWR

The distribution of all taxa should be mapped at a fine-scale with the boundaries of existing protected areas and the threatened status of each taxon should be determined. There is also a need for a red list threatened *Musa* CWR.

Traditional knowledge and uses linked to all wild *Musa* should also be recorded, mapped, stored and made accessible. According to the “crossed maps”, development of new protection measures could be proposed on a case-by-case basis.

For highly endangered taxa, specific agreements should be developed between concerned countries and international institutions to allow the efficient and permanent safeguarding of these resources. International banana research networks should work with national or regional research institutes to carry out a complete and up to date compilation of biodiversity distribution and conservation status.

8.3.2 On-farm conservation

Regional databases of *Musa* landraces should be developed (with characterization and evaluation data, indigenous knowledge, digital photo databases, and geo-referenced locations). From this data, distribution maps of landraces of geographic patterns for analysis can be made.

Identification of geographic specific traits should be carried out, i.e. which traits are specific to given areas (and their environmental constraints). Also, as with *in situ*, a red list should be made to allow the development of actions for their safeguarding landraces.

Efforts should be made to promote the on-farm cultivation of *Musa* diversity to face climate and other changes through agricultural resilience. There is a need for micro-evolution studies and to correlate natural and human selections to identify genes of interest, emphasizing criteria of selection by farmers and reasons for long term cultivation. Along with this is the need to identify and promote the cultural value of local landraces.

International banana research networks should work together with national or regional research institutes at enhancing the local capacity for biodiversity information gathering and analysis.

From the 2014 Bioversity literature review: The need for a sustainable on-farm conservation programme requires the following actions:

- It is recommended that efforts for on-farm management are first of all made in Southeast Asia. One of the countries with the most information available is India. An on-farm management project could be developed in different areas of the country, where *Musa* cultivars and wild species are both used by communities. The report also suggests that attention must also be placed in other countries such as Vietnam, Indonesia and Papua New Guinea
- Explore the policy and procedural changes required at different levels to encourage the conservation and management of on-farm landrace diversity
- Promote access to planting materials of cultivars and establishment of farmers' networks to facilitate propagule exchange
- Identify characters and qualities of landraces seen by farmers and consumers as inadequate
- Increase access to markets or create new preferential markets for landraces
- Promote agro-ecotourism to raise awareness of the benefits of growing locally adapted landraces.

For these actions to take place, the following areas are proposed for further research:

- Study through geographical information systems (GIS) where different cultivars are grown on-farm and how they have changed overtime so that genetic erosion is monitored and a tool can be developed for territorial monitoring of on-farm diversity
- Apply GIS studies to understand and map the conservation status of different populations of *Musa* landraces in secondary centres of diversity and to allow research on rare cultivars
- Study the social, economic and cultural aspects of traditional *Musa* farming systems to understand why, how and when farmers grow landraces so that they can be supported in conservation strategies.

Currently, few links exist between *ex situ* conservation and on-farm conservation activities and information on traditional knowledge and uses linked to landraces conserved *ex situ* is often missing. The result of such a situation is that the germplasm conserved *ex situ* is likely to be largely under-exploited, and doesn't answer the particular needs of farmers' communities.

On-farm conservation of landraces often meets the needs of local farmers and thus may serve as a critical and cost-effective conservation tool within a large-scale conservation and use strategy. The approach is low cost but not safe. A better understanding of the constraints and opportunities of on-farm conservation is important to underpin its value in this strategy and thus further study is recommended.

In addition to the above recommendations, the Bioversity 2014 literature review presented a methodology proposal for future on-farm management projects. The methodology harmonizes the peculiarities of the genus *Musa* with the available literature on general methodologies for on-farm projects. It highlights:

- The key role played by farmers in any on-farm management project
- The importance of supportive policies to guarantee the sustainability of the project
- The central role played by women in conservation of *Musa* landraces and as keepers of indigenous knowledge
- The importance of carefully adapting all the interventions to the specific context
- The importance of impact assessment once the activities have been implemented. The project should not finish when the interventions have been completed, but with the long-term evaluation of the success of the activities at household, community and regional level.

The section below summarizes the four phases of the proposed on-farm management methodology.

As stated in the 2014 Bioversity literature review, all the phases must be adapted to local characteristics. The methodology must be implemented in different countries for testing and refining and also to check how local characteristics influence the development of the project. Finally, monitoring and impact assessment are essential to assess the real effect of the project on *Musa* diversity.

Phase 1: Defining Banana Diversity Areas

- Information on *Musa* diversity: potential sites
- Prioritization process: potential partners and stakeholders
- Assess sustainability: define ultimate objective.

Phase 2: Current Situation Definition

- Informal surveys (Participatory Rural Appraisal): sites and partners
- Formal surveys: Baseline - definition of current situation
- Assess need for action: Threats for diversity.

Phase 3: Development of an on-farm conservation strategy, monitoring strategy and implementation

Current situation – before intervention

- Improve availability of materials
- Improve information and availability of information
- Improve traditional cultivar materials and their management
- Improve processing
- Ensure the quality of planting material.

Post-intervention situation

- Market creation and market promotion
- Build partnerships and trust
- Change norms
- Promote ecological land management practices
- Payment schemes for ecosystem services.

Assessment and monitoring

- Track progress to guarantee meeting milestones and targets (i.e. ultimate objective).

Phase 4: Impact assessment and dissemination

- Evaluation of changes inside and outside sites/communities
- Crop diversity maintained or increased
- Public benefits - maintain and generate evolutionary services
- Private benefits - improve well-being.

Table 8.2 below summarizes the proposed actions concerning in situ and on farm conservation practices discussed in this section.

Table 8.2. *Objectives and proposed actions for* Musa *in situ and on farm conservation.*

Objectives	Proposed actions	
	In situ conservation of *Musa* CWR	**On farm conservation**
Map the distribution of CWRs and landraces in primary and secondary centers of diversity (potential sites - South East Asia (India) and the Pacific Islands)	• Establish and map the distribution of all taxa at all scales • Determine the threatened status of each taxon and red listing of highly endangered CWRs • Collecting and sharing traditional knowledge and uses linked to all wild *Musa* species.	• Build regional databases of *Musa* landraces (with characterization and evaluation data, indigenous knowledge, digital photo databases, and geo-referenced locations) • Develop distribution maps of landraces for analysis of geographic patterns • Identify geographic specific traits, i.e. traits which are specific to given areas (and their environmental constraints) • Establish and map the distribution of *Musa* landraces and farmers varieties • Determine the conservation status of all landraces and red listing those highly endangered.
Establish institutional frameworks for the conservation of CWR and *Musa* landraces	• Develop national and International Agreements to allow for efficient and permanent safeguard of CWRs in protected areas • Strengthen linkages with National and International *Musa* Research conservation networks • Develop a territorial monitoring tool for CWR diversity conservation.	• Develop national and International Agreements to allow for efficient and permanent safeguard of landraces in primary and secondary centres • Facilitate national, regional and international networks to enhance local capacity for biodiversity information gathering and analysis • Develop a territorial monitoring tool for landrace on farm diversity conservation.
Promote farm conservation and utilization of landraces under changing climatic conditions.		• Identify and promote the cultural value of local landraces • Facilitate national and international agreements on the conservation and use of landraces.
Collect and establish DNA/RNA bank for all major landraces		• Carry out studies on genomics and associated trait characterization • Evaluate landraces against major stresses (drought, pest and diseases).

PART D

USE

Part D outlines the use of *Musa* germplasm in four chapters: Chapter 9 focusing on *Information Management*; Chapter 10 on *Distribution and Safe Exchange of Germplasm*; Chapter 11 on aspects of *Musa Evaluation* and Chapter 12 on *Genetic Improvement*.

CHAPTER 9. *MUSA* GERMPLASM INFORMATION MANAGEMENT

SECTION 9.1 INFORMATION MANAGEMENT – WHERE WE ARE NOW

Taxonomic experts, breeders and other researchers attribute much of plant genetic resources under-utilization to the lack of availability and accessibility of useful information associated with specific germplasm. Conservation, analysis and use of genetic resources rely partly on information systems describing germplasm held in collections. However, numerous *Musa* collections have not been systematically documented and only limited amounts of characterization and evaluation data are currently available and remain scattered across multiple research sites and/or institutions. This lack of information prevents curators and other users from rationalizing collections, identifying specific accessions, understanding general characteristics of subgroups and optimizing the use of *Musa* diversity.

Despite all efforts and progress realized in past years, there is still a need for more quality information on taxonomy, characterization, evaluation, health status, availability, performance and usefulness, which is well captured at source, i.e. in the field genebanks or with new accessions acquired from another collection. Complete passport data are essential to verify the identity of the materials. Without this, conservation and use is pointless, as the value of the material cannot be known and the information cannot be accessed or shared. Similarly, without clear records on the use of the material, it is impossible to monitor and evaluate the usefulness of a collection and thus, to justify the considerable costs incurred and investments made in its management.

Therefore, documentation of germplasm is essential and should be a core activity of all genebanks. Individual collections need local germplasm management systems documenting each step of the conservation process, from acquisition to distribution, including regeneration and health testing. Guidelines and tools for documentation are important to facilitate the management of collections and to provide a common language for exchange of germplasm information. At the same time, a dedicated crop registry stimulating research and community curation is required to select material based on relevant data ideally from all the existing collections.

In order to monitor current practices in *Musa* germplasm documentation and better understand the needs and constraints relating to information management activities, the Global *Musa* Survey carried between 2012-2016, with replies from 56 genebanks curators, serves as a baseline for this strategy document.

9.1.1 *Ex situ* Collections Data Management

Monitoring of the different conservation activities such as acquisition, maintenance, rejuvenation, distribution and health testing is critical for the local management of any collection. The use of data management systems in collections has increased over the years but much still needs to be done to improve their use and potential. Collections maintain valuable materials but efficient ways of accessing information and the material are often lacking. Data associated with passport information, characterization, evaluation, management, shipment and photos is recorded using a variety of tools ranging from database systems, spreadsheets, paper such a log books or combinations of these. Using electronic documentation tools ensures that the data is stored electronically and therefore can be easily secured and exchanged.

According to the survey, most of the data capture is done using spreadsheets (e.g. MS Excel) and some captured using paper only to record germplasm information. Relatively few collections have adopted a database system to handle all information, with the majority using a combination of electronic tools. The proportion of data available in electronic format however varies across data types (Table 9.1). Passport data (e.g. accession name, country of origin) is documented in a database and/or spreadsheet in 60% of genebanks. In 17% of cases, it is available on paper only, and 4% do not collect this data at all. Management data (date of acquisition, health of material, regeneration status etc.) is documented using databases and spreadsheets in 33% of genebanks and using paper only in 35% of cases. Regarding characterization data, it is documented using databases and spreadsheets in 55% of collections and paper only in 24%. Similarly but in a slightly higher proportion, evaluation data is documented using databases and spreadsheets in 62% of genebanks and paper only in 17%. This may reflect the fact that evaluation data is often recorded for specific projects where it is mainly recorded using a spreadsheet (47%). Furthermore, information on distribution and shipment is not collected at all in 35% of collections. This may be due to a low rate of distribution of the material, which is alarming, and/or to the lack of tracking requests and use of the material for potential feedback.

Table 9.1. *Tools used for the management of the data on the germplasm collections.*

	Photographs	Passport data	Characterization data	Evaluation data	Management data	Shipment data
Database only	12%	11%	6%	4%	11%	5%
Spreadsheet only	33%	41%	37%	47%	33%	25%
Spreadsheet/ Database	4%	8%	12%	11%	9%	3%
Spreadsheet/ Database/ Paper	4%	8%	4%	2%	2%	5%
Database/Paper	2%	0%	8%	9%	2%	2%
Spreadsheet/Paper	4%	11%	10%	9%	7%	10%
Paper only	31%	17%	24%	19%	35%	15%
Not collected	10%	4%	0%	0%	2%	35%

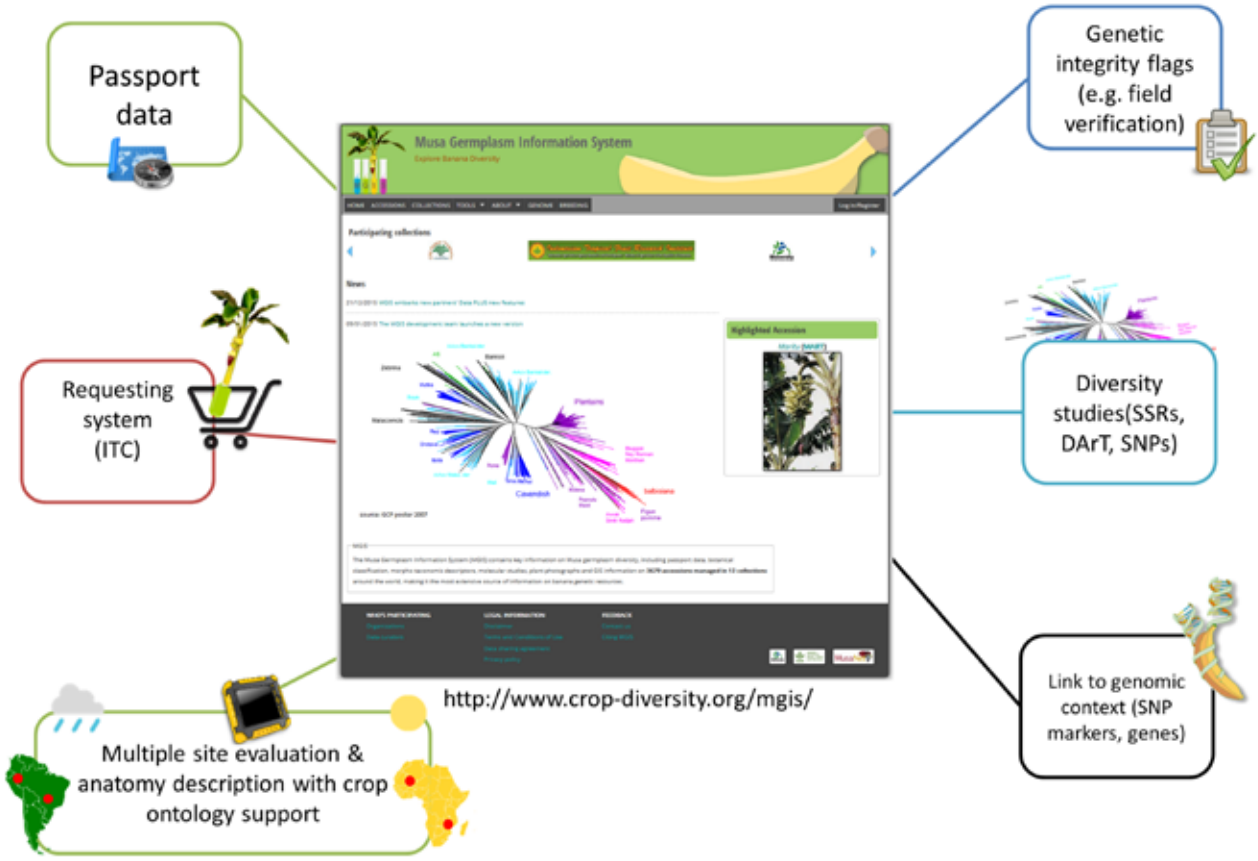
Regarding access and availability of the information related to germplasm, according to the survey, in most cases, the data is publicly available to all (33%), as published catalogues (31%) or upon request as electronic downloads (47%). In 24% of collections, data is available on institutional websites at all times. In some cases, if the data is not publicly available, it is still available upon request or through project reports.

The survey also highlighted the lack of effective technology to document and manage collection information digitally and the pressing need to support the deployment of a user-friendly genebank documentation system in many *Musa* collections. The survey identified a list of tools currently in place in some collections such as at ITC (i.e. MGBMS - *Musa* genebank management system), IITA, CNRA, INIVIT, CRB, BARS, SPC and NARO. However only a few of these are specifically designed to manage genebank collections and others are adapted but have limited functionality. Several collections are seeking new or upgraded data management systems. With this objective in mind, the GCDT joined forces with USDA/ARS and Bioversity to propose GRIN-Global, a new scalable version of the Germplasm Resource Information Network (GRIN) used by the USDA/ARS National Plant Germplasm System and suitable for use by any interested genebank. The database is deployable on local stand-alone computers at sites with limited computational capabilities, as well as at networked sites. This approach enables GRIN-Global to serve either centralized or decentralized genebank networks and to share data with third parties.

9.1.2 The *Musa* Germplasm Information System - MGIS

Since 1997, the *Musa* Germplasm Information System (MGIS: http://www.crop-diversity.org/mgis/) has been developed, with the objective of collecting and sharing publicly available information for all *Musa* collections worldwide. It contains key information, including passport data, botanical classification, morpho-taxonomic descriptors, ploidy, somatic chromosome number, plant photographs, geo-referenced information and genetic studies based on molecular markers. The main target users are: 1) germplasm curators requiring a global system for data sharing, comparison and sometimes to manage data pertaining to their own collection; 2) researchers, breeders and direct users of the germplasm selecting the most documented material for various types of experiments and other uses, and 3) general users looking for reference information on cultivars and a summary of their characteristics and uses. Overall, MGIS helps users build customized queries, facilitates data upload and quality control, locates alternative sources of banana germplasm and identifies the most appropriate accessions with particular traits of interest (Figure 9.1).

Figure 9.1. *Features and functionalities of MGIS released in January 2015.*



MGIS started as a CD-based tool until the release of the first online version in 2003, followed by two major updates in 2009 and 2014. MGIS has been designed for managing accession-level information using commonly agreed descriptors for characterization and agronomic evaluation. In order to facilitate and standardize the description, banana accessions were documented using standards such as the MultiCrop Passport Data (MCPD) and the *Musa* Descriptors published as a comprehensive booklet in 1996 (IPGRI 1996). Shorter descriptor lists, extracted from the 1996 booklet, provided an initial strategic set of characterization and evaluation descriptors to facilitate access to and utilization of banana accessions held

in genebanks (see Chapter 5 – *Characterization*). Furthermore, an electronic collecting form linked to MGIS has been developed to rationalize the data upload and avoid redundancy in collections.

As of January 2016, MGIS maintains information on 3,630 accessions from 11 collections (plus 3,091 historical accessions from 16 collections conserved as archives). The number of collections and data volume are both expected to grow steadily with the signing of Data Sharing Agreements (DSA) with partners' collections (Table 9.2). The partnerships and collaborations are indeed at the heart of the MGIS *modus operandi* for collecting and exchanging data. The collaboration ratified by co-signing the DSA, whereby both parties commit to deliver the most accurate information available of *Musa* germplasm. The MGIS DSA is found in Annex E.

The DSA specifies the responsibilities of the recipient (i.e. Bioversity) responsible for its management and of the data providers as the owners of the data.

The data providers (i.e. *ex situ* collections) agree to:

1. provide the data in an agreed format for Bioversity to upload to MGIS
2. obtain all permissions and licences from third parties, including in relation to copyright and database rights, to allow the data to be made publicly available on MGIS
3. provide Bioversity only non-confidential data that is not subject to any restrictions
4. update their data at least once a year

Bioversity, as MGIS manager agrees to:

1. provide public access to MGIS via a website, and maintain this website and its user interfaces
2. not alter or modify the data, except for resizing of photos
3. acknowledge the source of the data and not claim any copyright or other intellectual property rights
4. pass on the same restrictions to the users in the form of a Terms of Use Agreement
5. make the data available to global information systems including GENESYS, the Global Information System for Plant Genetic Resources for Food and Agriculture (as per Article 17 of the International Treaty on PGRFA) and the Global Biodiversity Information Facility (GBIF), and ensure that the data providers are appropriately acknowledged

As specified in the DSA, MGIS provides quality data to GENESYS, reflecting the work done by curators and experts within the framework of MusaNet to ensure a better, more relevant and valuable *Musa* documentation.

Table 9.2. *List of banana collections and status of data sharing in MGIS (as of November 2015).*

Collections in MGIS (DSA signed and data updated)			
Country	**Acronym**	**Institute**	**No. of accessions**
Australia	DAFF South Johnstone	Department of Agriculture, Fisheries and Forestry, Queensland Government	42
Belgium	ITC	Bioversity International *Musa* Germplasm Transit Centre	1505
Cameroon	CARBAP	Centre Africain de Recherche sur Bananes et Plantains Station de Recherches Agronomique	365
China	IFTR/GDAAS	Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences	217
Costa Rica	CORBANA	Corporación Bananera Nacional S.A.	108
France (Guadeloupe)	CRB-PT	Centre de Ressources Biologiques Plantes Tropicales CIRAD-INRA	354
Papua New Guinea	NARI-LALOKI	Dry-lowlands Research Programme, Laloki	62

Collections in MGIS (DSA signed and data updated)			
Country	**Acronym**	**Institute**	**No. of accessions**
Puerto Rico	USDA-TARS	United State Depart. Of Agriculture, Tropical Agriculture Research Station	152
Uganda	NARO	National Agricultural Research Institute, PGR Unit	442
Ivory coast	CNRA	Centre National de la Recherche Agronomique	65
Congo	INERA	Institut National pour l'Etude et la Recherche Agronomiques	57
Indonesia	ITFRI	Indonesian Tropical Fruit Research Institute	306
Collections not in MGIS (DSA signed but data not yet available)			
Country	**Acronym**	**Institute**	
Benin	INRAB	Institut National de Recherche Agricole	
Central African Republic	ICRA	Institut Centrafricain de Recherche Agronomique	
Gabon	CENAREST	Centre National de la Recherche Scientifique	
Ghana	CSIR-CRI	Council for Scientific and Industrial Research - Crops Research Institute	
Philippines	BPI	Bureau of Plant Industry	
Tanzania	ARI-Maruku	Agricultural Research Institute Maruku	
Vietnam	FAVRI	Fruit and Vegetable Research Institute	
Collections not in MGIS (DSA not signed)			
Country	**Acronym**	**Institute**	
Australia	DAFF-Mar	Department of Agriculture , Forestry and Fisheries, Maroochy Research Facility	
Brazil	EMBRAPA	EMBRAPA Mandioca e Fruticultura	
Burundi	IRAZ	Institut de Recherche Agronomique et Zootechnique	
China	TSFR Lab	South China Agricultural University	
Colombia	CORPOICA	Corporación Colombiana de Investigación Agropecuaria	
Cook islands	MoA	Ministry of Agriculture	
Cuba	INIVIT	Instituto de Investigaciones de Viandas Tropicales	
Ethiopia	EIAR-Jimma	Ethiopian Agricultural Research Institute, Jimma Research Center	
Ethiopia	EIAR-Melkassa	Ethiopia Institute of Agricultural Research, Melkassa Agricultural Research Center	
Fiji	SRS	Sigatoka Research Station - Ministry of Agriculture	
Fiji	SPC	Secretariat of the Pacific Community	
French Polynesia	SDR-FPNC	Service du développement rural, French Polynesia national collection	
French Polynesia	SDR-PRFC	Service du Développement Rural, Pacific Regional Field Collection	
Honduras	FHIA	Fundación Hondureña de Investigación Agrícola	
India	IIHR	Indian Institute of Horticultural Research	
India	NRCB	National Research Centre on Banana	
India	KAU	Kerala Agricultural University	
India	NBPGR	National Bureau of Plant Genetic Resources	
Indonesia	IIS-PBG	Purwodadi Botanic Garden – Indonesian Institute of Sciences	
Indonesia	IIS-RCB	Indonesian Institute of Sciences, Research Center for Biology	
Kenya	KALRO-KISII	Kenya Agricultural and Livestock Research Organisation	
Kenya	KALRO-Thika	Kenya Agricultural and Livestock Research Organisation	
Malawi	BARS	Department of Agricultural Research and Technical Services	
Malaysia	MARDI	Malaysian Agricultural Research and Development Institute	
Mauritius	AREU	Agricultural Research and Extension Unit	
Mexico	INIFAP	Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias	

Collections not in MGIS (DSA not signed)		
Country	**Acronym**	**Institute**
Nigeria	IITA	International Institute of Tropical Agriculture
Philippines	IPB/UPLB	National Plant Genetic Resources Laboratory
Philippines	UPLB	University of the Philippines at Los Banos, Department of Horticulture
Rwanda	ISAR	Institut des Sciences Agronomiques du Rwanda
Samoa	MAF	Ministry of Agriculture and Fisheries
South Africa	ARC-ITSC	Institute for Tropical and Sub tropical Crops of the Agricultural Research Council
Sri Lanka	HORDI	Horticultural Crops Research and Development Institute
Sudan	ARC - ITSC	Institute for Tropical and Sub tropical Crops of the Agricultural Research Council
Taiwan, province of China	TBRI	Taiwan Banana Research Institute
Togo	ITRA	Institut Togolais de la Recherche Agronomique
United States of America	Waimea	Waimea Valley Arboretum and Botanical Garden
Vanuatu	VARTC	Vanuatu Agricultural Research and Technical Centre

SECTION 9.2 INFORMATION MANAGEMENT - WHERE WE WANT TO GO

In order to support the core activities of collections, they should have access to appropriate solutions for efficient management of their routine operations. Therefore, it is essential to increase data quality, data completeness and facilitate data capture with modern tools and appropriate standards. This could be done by i) making available a set of tools that will facilitate an increase in data of the conserved genepool by providing a global view of the conserved genetic diversity and ii) focusing on a subset of accessions and providing a catalogue of accessions well-documented for agronomic traits and in different environments and genetic background; thus promoting distribution and use. In this way, MGIS, as a global crop portal, can be used as a pivotal and reliable information management system for *Musa* genetic resources.

Feedback on MGIS has been provided during a number of consultation meetings regarding users' expectations, improvements on data ownership, on usability and data quality. Below are the main areas of improvement:

- Many users require more information than just accession-level information. For example, users often need general taxonomic information to be able to search accessions. Information on specific cultivars can be obtained in Musapedia, which can easily be linked to MGIS
- MGIS should extend information on all *Musa* and include data from more collections
- MGIS needs to bridge the gap between molecular data and related analysis tools
- Data quality, reliability and validation should be improved by using tools such as the ones developed for the *Musa* Crop Registry to help identify cross-referenced accessions between MGIS collections
- The number of high quality photos with proper ownership credits should be increased
- Access to publications and references should be improved for verification of data in MGIS
- Incentives for collection curators to provide data could be promoted such as collaborative projects (e.g. the Taxonomic Reference Collection project)
- Acknowledgement and recognition of contributions is crucial. The data ownership could be improved by creating functional links between all collections, curators and MGIS to encourage the provision of clean data (both for data providers and for MGIS), including clear data attribution and recognition

- MGIS can help in networking all collections' databases. Regular contacts and communication between MGIS and the collection curators should be strengthened to motivate data providers and improve data quality in a sustainable way
- Feedback to and from MGIS should still be through personal contacts with MGIS and the collections managers and not through automatic systems. Users need to know the person behind the system and the source of information to interact more actively
- MGIS must facilitate access to evaluation data.

The overall objective is to ensure that the data collected through MGIS and linked *Musa* databases are reliable enough, freely accessible and downloadable, thus becoming the main source of information for *Musa* genetic resources. An effort should be carried out to simplify the flow of data being shared and to facilitate the exchange of data between collections. MGIS should also facilitate importing/exporting data from/into electronic spreadsheets and printing summary information on accessions (similar to cultivar cards in the Musalogue catalogue (Daniells et al. 2001).

Special attention and efforts are required to ensure that collection curators receive support, in a timely and effectively manner, to ensure a better documentation of their collection. As an example, training sessions were organized for such purpose during three recent MusaNet workshops (at CIRAD, Guadeloupe in 2013, at NRCB, Trichy in 2014, and at CARBAP, Cameroon in 2015). Training material for MGIS and Grin-Global needs to be disseminated including on-line help and video demonstrations. Finally, increased visibility and acknowledgement of curators and data providers is critical for encouraging data sharing and research collaboration. National and regional networks are important and can assist in stimulating dialogue to convince heads of national research institutions to share data on *Musa* germplasm.

SECTION 9.3 INFORMATION MANAGEMENT - HOW WE WILL GET THERE

Data quality and completion can be greatly enhanced by training and supporting collections with tools and standards. The release of a new version of MGIS desktop application supporting the exchange of data combined with development of user-friendly mobile applications for data capture can help reach this objective. Moreover, image search recognition technology will be investigated for computer-assisted identification as well as the use of ontologies to ensure annotation of agronomic data in a standardized way. Finally, comprehensive documentation will be reached by integrating several layers of scientific knowledge generated in advanced labs, greenhouses and in fields by genebank curators, molecular biologists and breeders. Dealing with the wealth of molecular and phenotyping data and enabling interoperability between information systems will offer a platform of reference for the documentation of banana genetic resources.

9.3.1 Improving data quality and accuracy

Efforts are continuously made to improve data quality in MGIS and MusaNet members are collectively working to improve the quality of the data at the collection level. As an illustration, workshops have been organized to train curators on the use of MGIS and to share experience on characterization and germplasm management. MGIS has undergone a number of revisions to respond to the feedback gathered during these workshops. MGIS was recently expanded to include representative sets of pictures for the characterization of the accessions. It will facilitate and encourage the use of MGIS data and photos by allowing the online creation of Musalogue-like cards (Arnaud and Horry 1997).

It is therefore important to ensure that curators receive appropriate guidelines, tools and training. As a first step, MusaNet published guidelines for taking the minimum set of photos (TAG 2010). This approach requires a closer relationship and collaboration between curators and information system managers to ensure that high quality data is provided. It is important to acknowledge that a mechanism to improve quality of the data in MGIS will rely on curators, who play a crucial role in achieving improved documentation. Also, increasing the amount of collection data in MGIS should lead to an improved,

effective, and user-friendly information system with cross-references, as exists for rice and wheat (Hazekamp et al. 2014).

Indeed, MGIS can support rationalization, gaps analyses and identification of potential inconsistencies in passport data (e.g. misclassification). This has never been achieved, mainly because of lack of input from experts who required appropriate data, in particular a set of quality pictures. There is a need for a group of experts to provide oversight not only for the taxonomic information but also for the development of the content of the database. Addressing the information needs of germplasm users requires strategic thinking in terms of data gathering, quality control, analysis and provision. Data quality scrutiny of the MGIS database content should be regularly performed and facilitated by standardized workflows. Accessions with limited data should be particularly analysed to identify additional information that could be included (e.g. presence of seeds in a bunch). There is also a need to confirm certain accessions, especially those only known by local name as discussed in the ITC impact review (Garming et al. 2010).

As a result, germplasm datasets will be updated and cleaned following published literature on improving and managing the quality of passport data maintained in databases (van Hintum et al. 2011) and in partnership with curators of the collections. Additionally, the completion of passport data from individual collections will be scrutinized prior to inclusion into the MGIS database to improve the Passport Data Complexion Index (PDCI) and the same will apply for characterization and evaluation data. MGIS will provide more information on the geographical sites of the collections and facilitate the exchange of information between collection curators. It can promote the use of germplasm from specific collections if it contains information on its availability. This standardized detailed examination will allow the *Musa* community to feel confident about the credibility of the data included in MGIS for any subsequent analyses.

Apart from guidelines, one way to support data quality is to promote the use of mobile devices (e.g. field books) for gathering characterization information in an electronic format, which should facilitate the efficient collection, management and the publication of data. MusaNet's Diversity Thematic Group will support the development of such tools to better document *Musa* spp. genetic resources. A final version of the mobile device application, MusaTab, tested at recent MusaNet workshops, will be freely available in 2016. The further development of such devices is strongly recommended as it not only facilitates the data recording but provides integrated tools such as a camera to take photos associated to specific descriptors as well as training videos on how to interpret specific character descriptors.

A potential application of this approach would be cultivar recognition and identification based on digital images collected during field verification evaluations of ITC accessions or germplasm descriptors. Another potential application could be the ability to perform GIS analysis on geo-referenced *Musa* spp., with their corresponding characterization information using raw data downloaded from the MGIS database. Synergies can be established with international initiatives like Pl@ntNet (Joly et al. 2014), who are developing software to compare botanical pictures against a database in order to help in the identification process. In 2011, Pl@ntNet developed a prototype with a set of pictures extracted from MGIS representing the different states of seven descriptors. It raised the need for more and better quality photos which is currently being addressed with the guidelines and development of the mobile application. Based on the updated descriptors list for banana and datasets, a revitalized version of Musa.ID (Perrier and Tézenas Du Montcel 1990) is proposed to support taxonomical identification with probabilistic models. The release of the new version is planned for May 2016 and will be available for download from MGIS.

9.3.2 Linking and complementing datasets

The ITC code (the number that identifies an accessions in the ITC global collection) is widely used and it should become standard practice to quote it for any germplasm under research and related publications. The accession code from the original collection should also be quoted, especially when the material is not present in the ITC collection. This is a pre-requisite for fostering interoperability between diverse resources on banana genetic resources.

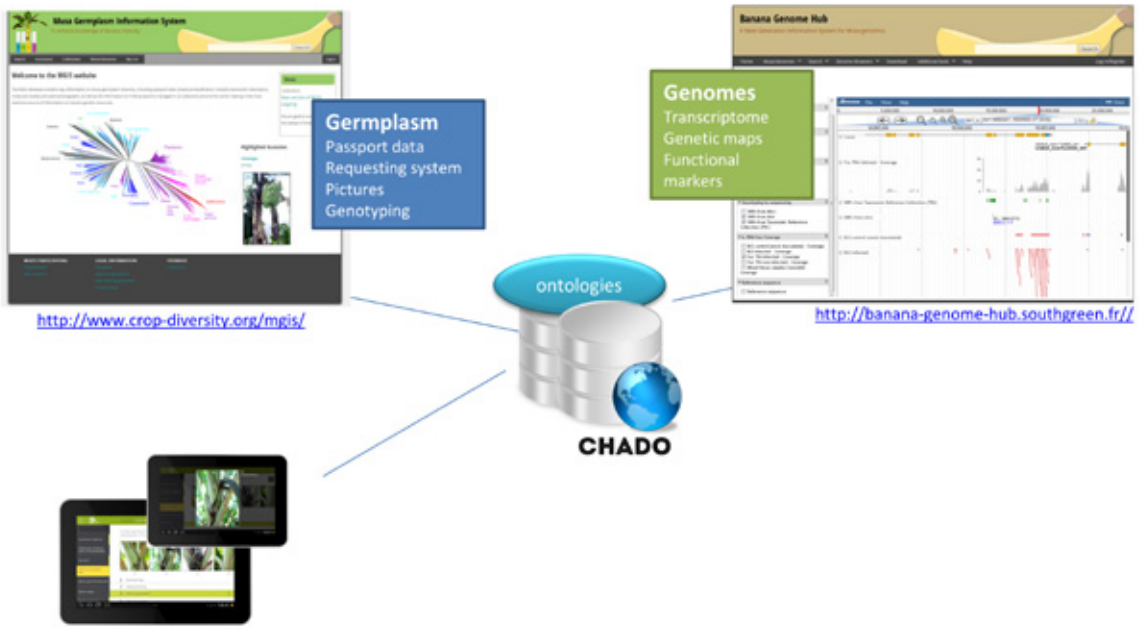
9.3.3 From accession level to cultivar level

Breeders need information related to fertility, post-harvest qualities and use of cultivars elsewhere, which is not available in MGIS. The taxonomic reference set of cultivars may provide a starting point for gathering complementary data on quantitative traits, uses, etc. For instance, it has been proposed to provide information on the importance and value of the cultivar, including commercial and indigenous knowledge, post-harvest fruit quality, pests and diseases and other agronomic characteristics and performance. For that purpose, MGIS should be linked to other information resources such as Musapedia (http://www.promusa.org/Musapedia) and the *Musa* Literature database, MusaLit (http://www.musalit.org/).

9.3.4 Genomics of genebanks

As for many other crops, insufficient genetic information is available about the holdings in genebanks. However, large-scale genetic and phenotypic characterization of germplasm collections has great potential to change the way scientists deal with genetic resources (McCouch et al. 2012). Massive amounts of sequence data are also being generated in banana (Hueber et al. 2015) and users of genebanks should be in a position to select germplasm material based on a combination of passport, genotypic and phenotypic information from the global genepool. A new generation of information systems needs to be designed to efficiently handle this information and link it to external resources such as genome databases (Guignon et al. 2015). The latest version of MGIS includes genetic studies based on genotyping with microsatellite markers and single-nucleotide polymorphisms (SNPs) from Genotyping-By-Sequencing (GBS) on several hundred accessions. Taking advantage of those datasets, we can strengthen interoperability between genetic resources and the genomic context available (D'Hont et al. 2012; Davey et al. 2013; Martin et al. 2016) and managed in the Banana Genome Hub (Droc et al. 2013), as illustrated in Figure 9.2. The Banana Genome Hub contains the Musa acuminata genome of reference as well as related datasets such as gene-expression data coming from various publications related to biotic or abiotic stress and fruit quality (e.g. Li et al. 2012; Bai et al. 2013; Jourda et al. 2014; Lee et al. 2015; Zorrilla-Fontanesi et al. 2016). It will pave the way to identify chromosome regions or genes involved in important agricultural traits (e.g. genome-wide association studies (GWAS)) to better understand mosaic genome structure, structural variations and functional gene diversity between accessions. Further options will be explored in consultation with the MusaNet Genomics Thematic Group (GTG).

Figure 9.2. *Example of data interoperability between genetic resources (i.e. MGIS) and genomic resources (i.e. Banana Genome Hub).*



9.3.5 Phenotyping and evaluation

Evaluation trials are often performed through specific projects beyond a genebank's remit, but very often the germplasm used in these trials comes from the genebank, and therefore it is beneficial for the collection to connect these data to accessions held in the collection. This linkage ensures a better and more exhaustive documentation oriented to the use of germplasm. Until now, evaluation data has been stored in MGIS as observations made by curators in their collections but not explicitly linked to the accessions. A first attempt was made with IMTP data that were stored in the Global Agricultural Trial Repository (http://www.agtrials.org), but it unfortunately has limitations for interoperability.

With an increasing amount of phenotypic information available, one of the key limiting factors in their use is the lack of standard nomenclature used to describe crop development and agronomic traits. Plant breeders measure several traits to understand the phenotype, based on genotype and environment variations. In order to facilitate access to data held in a range of databases and facilitate the harmonization of the data, we will use a crop ontology portal that provides access to multi-crop passport data, anatomy, development stages, and agronomic traits (Shrestha et al. 2010; 2012). The ontology is a harmonized and structured list of traits, names, methods and scales of measurement that can be used in databases like MGIS to handle evaluation data (Van den Bergh et al. 2014). Ontologies can easily be uploaded or created online, which encourages partnerships for the cross-referencing of terms. However, ontologies for *Musa* are still in their infancy and will require further support from the MusaNet ETG. This relates to the integration of phenotypic, genotypic and environmental data associated with a given trait.

Table 9.3. *Summary of objectives and proposed actions related to* Musa *germplasm information management.*

Objectives	Proposed actions
Provide a set of tools to improve data quality and accuracy and facilitate data-capture in collections with modern tools	• Release mobile application for data capture in the field • Implement indicators of data completion for passport and characterization data • Provide regular training using the latest tools.
Sustain development of a banana community portal for genepool banana diversity	• MGIS regularly updated with new collection and data • Link the accession level to cultivar level to retrieve information on the importance and value of the cultivar, including commercial and indigenous knowledge and potential post-harvest fruit quality, pests and diseases and other agronomic characteristic and performance.
Complement documentation of accession with phenotyping and evaluation data and facilitate data harmonization	• Record agronomic traits data from evaluation studies on germplasm material • Harmonize data with crop ontology for multi-crop passport data, anatomy, development stages, and agronomical traits.
Embrace the genomics of genebanks and aggregate omics data generated from germplasm material held in collections	• Banana Genome Hub regularly updated with new datasets • Interoperability fostered between MGIS, Banana Genome Hub and breeding resources such as Musabase.

CHAPTER 10. DISTRIBUTION AND SAFE EXCHANGE OF GERMPLASM

SECTION 10.1 DISTRIBUTION AND SAFE EXCHANGE - WHERE WE ARE NOW

10.1.1 Distribution of Germplasm

The main source of *Musa* germplasm available for distribution internationally is the ITC, which holds the most comprehensive set of diversity that can be obtained from a single source and for which the health status is guaranteed (see Chapter 7 – *The ITC Global Musa Collection*). However, only small sample sizes can be provided by the ITC. National repository and dissemination centres are therefore necessary to meet the demand for germplasm. For example, in the Asia-Pacific area, 13 national centres and one regional distribution centre were established (at the Secretariat of the Pacific Community (SPC), in Fiji), but this additional capacity is still insufficient to meet users' needs.

The stakeholders directly affected by the distribution and safe exchange issues addressed here are the genebanking community, the governmental and non-governmental collections, botanical gardens, conservation scientists, managers and service providers who are responsible for the sustainable conservation of *Musa* genetic resources. Key users are the banana research community and networks (universities, NARS, IARCs, private sector) that need access to biologically well-defined material as a tool for generating new knowledge; and the banana breeders who need sources of genes, pre-bred material and associated information to develop superior cultivars. The ultimate beneficiaries, thanks to improved agricultural productivity, are the farming communities, production and processing industry and consumers. Other stakeholders in the system are policy makers and authorities regulating access to and exchange of genetic resources in accordance with treaty obligations and local, national and international phytosanitary legislation.

10.1.1.1 Agreements for the International Exchange of Germplasm

An important constraint to germplasm use is the difficulty faced by many scientists in obtaining materials from national collections from other countries. Policy and legal restrictions on access create bottlenecks and hinder efforts to ensure that genetic diversity contributes to improving cultivars in order to strengthen food security for the hundreds of millions of people who depend on them. Furthermore, every country is dependent on genetic resources from other countries for their own needs.

To address the fair sharing of benefits arising from the use of crop genetic resources, the ITPGRFA came into force in 2004 with currently 146 countries as contracting parties and signatories. The objectives of the ITPGRFA are the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of benefits derived from their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security. The ITPGRFA promotes international cooperation and open exchange of genetic resources, essential for food security. Through the ITPGRFA, countries agree to establish an efficient, effective and transparent Multilateral System (MLS) to facilitate access and share the benefits arising out of their use in a fair and equitable way. The MLS includes 64 crops and forages (referred to as Annex 1 crops). All *Musa* species except *Musa textilis* are included in the Annex 1 of the ITPGRFA. In addition, all collections maintained by the CGIAR including the ITC at Bioversity and IITA are included in the ITPGRFA under the Article 15b.

The Governing Body of the ITPGRFA has set out the conditions for access and benefit-sharing in a Standard Material Transfer Agreement (SMTA). The SMTA should be signed for each *Musa* sample that is sent from collections in the public domain of countries signatory to the ITPGRFA and received by any users. The SMTA facilitates the tracking of germplasm use, it ensures that the material remains in the public domain and indicates the rights and obligations linked to the reception of germplasm. The main benefits of sharing germplasm are the enhancement of improvement programmes, the sharing of information and technologies, and capacity building.

Most requests for germplasm from national collections require a formal approval from the institute's administration and the signing of a material transfer agreement (MTA) (an SMTA if the material falls under the ITPGRFA). In addition to an MTA, users may also have to obtain import permits and phytosanitary certificates to obtain germplasm. In many countries, the process for approving a request may involve high levels of government, which can create delays in responding to and fulfilling the request.

Out of the 56 *Musa* collections surveyed in the Global *Musa* Survey, 67% confirmed that their material is available for distribution outside of the country, whereas 33% of collections maintain germplasm that is not available for distribution outside the country. Only 25 of the 56 institutes use an SMTA for distribution and 4 institutes use an MTA developed by their institute for bilateral agreements. For the ITC collection, all accessions are part of the ITPGRFA and therefore exchange of germplasm occurs upon acceptance of the terms and conditions of the SMTA by the requestor. Only germplasm that is tested virus-negative is available for distribution.

10.1.1.2 Distribution of materials to different users

Musa germplasm can be distributed in the form of suckers, corms or *in vitro* plantlets as clonal propagation material. Information on the average number of accessions distributed per year was provided in the Global *Musa* Survey by about 35 collections. However, the data are difficult to analyse as some collections may have interpreted accessions and number of plants as the same and therefore numbers vary greatly, from 0 to 10,500 per year. But several points are salient:

- A number of collections indicated that their annual distribution of materials to local users as well as users outside the region was very low and zero in many cases
- Few collections provided exact figures, most provided an estimate and many (20-30) did not provide any data
- A few mentioned that material is distributed for project-specific research and in these cases, many accessions are targeted.

Figures 10.1 and 10.2 below indicate the average number of accessions (Y axis) distributed per individual collection (X axis) per year to local users and within the country. The distribution of accessions outside the country is very low and is therefore not illustrated.

Figure 10.1. *Average number of accessions (Y axis) distributed per collection (X axis) per year to local users – data displayed for 31 individual collections excluding the ITC.*

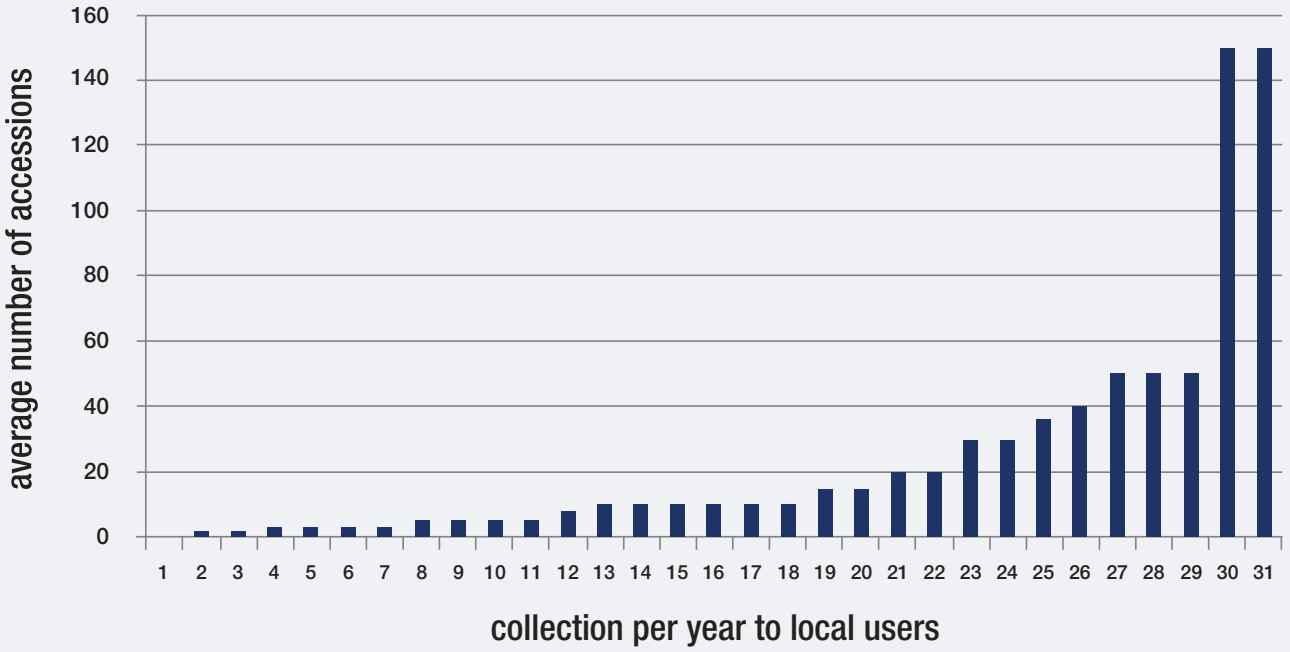
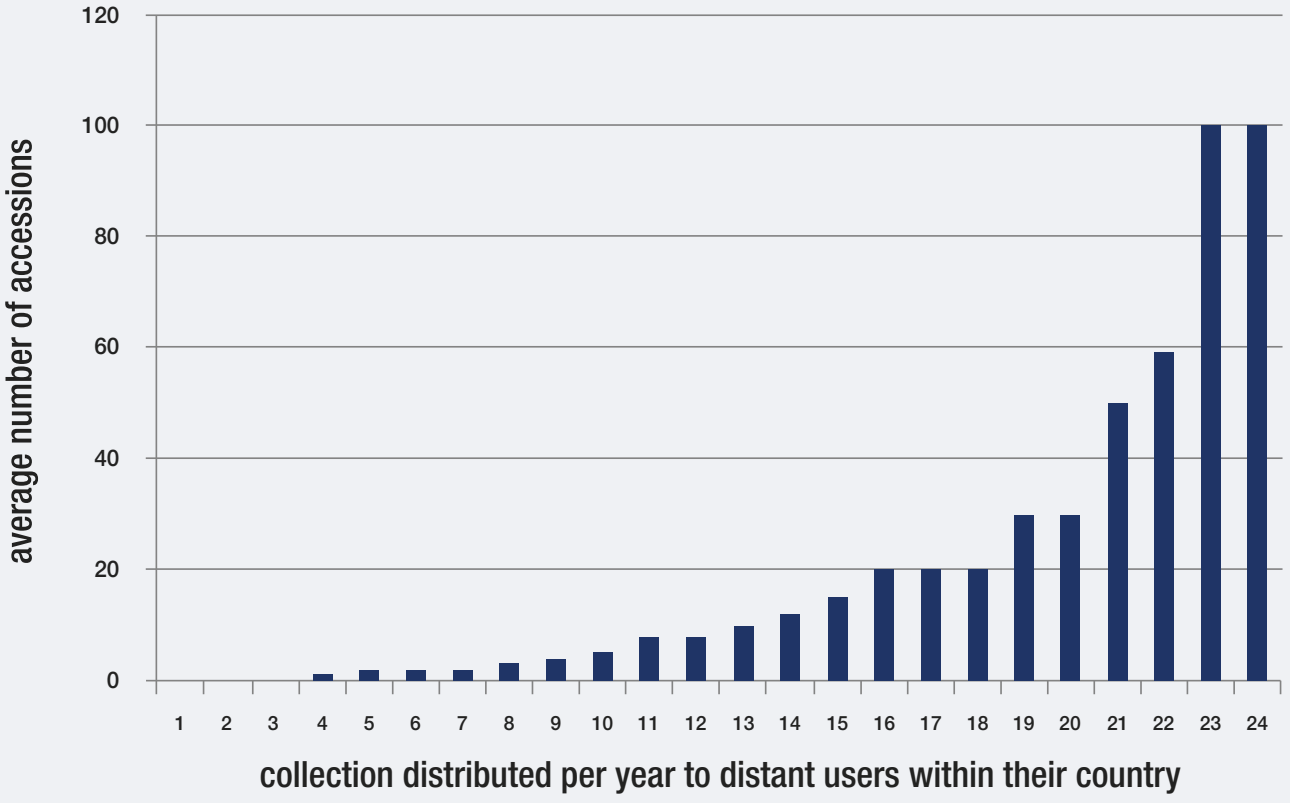


Figure 10.2. *Average number of accessions (Y axis) per collection (X axis) distributed per year to distant users within their country – data displayed for 24 individual collections excluding the ITC.*



The most frequent groups of external users of the *Musa* germplasm surveyed, in order, are researchers (rated as frequent by 80% of respondents), farmers (60%), the public sector (53%) and breeders (47%). The lower percentage use (based on requests) of the material by breeders can be due to the fact that globally there are very few *Musa* breeders. This was also reflected in the main purposes for the use of the disseminated materials mentioned, which are research activities (85% of respondents rated this as a frequent use), evaluation (75%), characterization (60%) and distribution to growers (67%), leaving pre-breeding and breeding to be rated a frequent use by only 30% of respondents.

The main purpose for the use of the disseminated materials, as reported by *ex situ* collections is for research activities (85%), evaluation (70%), distribution to growers (67%) and characterization (60%). The material is used for pre-breeding and breeding in 30% of the cases and for biotechnological research in 36% of cases.

The most important factors limiting the distribution and use of materials mentioned by the surveyed collections was the limited quantity of material available, material that is not certified disease free and lack of facilities/expertise to index the health status of germplasm. It is surprising to observe that documentation is not perceived by collection curators as a major limiting factor when this is regularly mentioned by users. See details in Table 10.1 below.

Other factors mentioned as major problems in the distribution and use of germplasm:

- *In vitro* activation of integrated BSV in B-genome accessions (see Section 10.1.2 below)
- Importation into some countries such as Australia can take two years to process before release Only virus indexed tissue culture plants can be legally moved across Australia
- Limited resources especially the human resources capacity in many countries
- Not enough researchers and financial support
- The complexity of the SMTA to receive ITC materials
- No request for some of the materials
- Not all accessions are of economic importance for adoption as cultivars.

Table 10.1. *Relative importance of factors limiting the distribution and use of materials from* ex situ *collections.*

Factors	Major factor	Minor factor	No effect
Legal restrictions/government policy	28%	32%	40%
Phytosanitary regulations (permits/certificates)	32%	40%	28%
Material is not certified disease free	49%	32%	19%
Limited quantity of material available	54%	35%	10%
Resources to prepare and ship germplasm	35%	35%	30%
Poor documentation status of germplasm	11%	39%	50%
Facilities /expertise to index the health status of germplasm	38%	34%	28%

10.1.2 Safe Exchange of Germplasm

For the conserved material to be exchanged, it first needs to be free of pests and pathogens including fungi, bacteria, viruses and insects. Accessions need to be carefully monitored during storage. Specific methods for detecting pathogens vary by organism and host, and protocols are required for accurate identification of most pathogens.

Viruses pose the major risk in the transfer of *in vitro* germplasm. The *Technical Guidelines for Safe Movement of* Musa *Germplasm 3rd edition* (Thomas 2015) describe technical procedures that minimize the risk of pest introductions due to the movement of germplasm for research, crop improvement, plant breeding, exploration or conservation. They are intended to provide the best possible phytosanitary information to institutions involved in small-scale plant germplasm exchange for research purposes. These guidelines are available online at www.musanet.org.

The safe movement of *Musa* germplasm requires adequate virus-indexing procedures. The currently recommended method is for a subset of tissue culture plantlets of an accession to be grown in post-entry quarantine for six months. Visual examination, molecular testing and electron microscopy is carried out at three and six months. Specific tests are undertaken for known viruses and non-specific tests for unknown viruses, and only plants indexed virus-negative are available for distribution.

The Global *Musa* Survey collected information on the following most damaging biotic agents affecting the *ex situ* field collections:

1. Fusarium wilt
2. BBrMV - Banana bract mosaic virus
3. BBTV - Banana bunchy top virus
4. BSV- Banana streak virus
5. CMV - Cucumber mosaic virus
6. Bacterial wilt
7. BLS - Black leaf streak
8. Other Mycosphaerella leaf spots
9. Nematodes
10. Weevils

Out of the 54 institutes surveyed, 27 have post-entry quarantine facilities either at their own institute or provided by a partner institute and 31 institutes have virus-indexing facilities or the service provided by a partner institute.

There are currently three *Musa* virus-indexing centres in Australia (University of Queensland), Belgium (University of Liège), and Nigeria (IITA), linked to regional collections. The role of a global virus-indexing centre is to test all germplasm deposited into a collection such as the ITC so that it can be distributed safely following the technical guidelines. The virus-indexing system through which all existing ITC accessions have passed (and new accessions will continue to be passed) provides a comprehensive mechanism to ensure that germplasm is, as far as possible, free of all viruses. At this time, about 60% of the ITC collection is virus negative and available for international distribution. For cost-effectiveness, a pre-indexing process that functions in concert with the formal virus-indexing centres is being undertaken at the University of Liège in Belgium.

Regional centres can also play an important role in facilitating regional exchanges. At the moment, however, procedures are not uniform across all regional centres and although there are acceptable alternative protocols, they must be approved and validated before being implemented.

10.1.3 Banana streak viruses - BSV

One major issue impeding the distribution of *Musa* germplasm, particularly from the ITC, is the presence of infective, endogenous banana streak viruses (BSV), which are integrated in the banana B genome, and can be activated to cause episomal virus infections under some circumstances. *In vitro* culture and hybridization are two common factors which can trigger activation and subsequent field disease.

Interestingly, after the thorough characterization of eBSV from the three main BSV species (Obino l'Ewai virus – BSOLV; Goldfinger virus – BSGFV; Imové virus – BSIMV) unearthed in the seedy *M. balbisiana* diploid Pisang Klutuk Wulung (PKW), the CIRAD team named "Biodiversity of endogenous and exogenous Badnaviruses" has developed specific PCR and derived Cleaved Amplified Polymorphic Sequence (dCAPS) molecular markers enabling the genotyping of infective and non-infective eBSV alleles of these three BSV species (Gayral et al. 2008, Chabannes et al. 2013). They also demonstrated that these alleles were strongly conserved among the Musa diversity (Duroy et al. 2016). These tools are and will be crucial to screen germplasm collection, genitors and segregating populations during breeding programs by markers assisted selection (MAS). Those markers have already been fruitfully used at CIRAD to generate, by self-pollination or duplication of chromosomes sets from haploid lines, some *M. balbisiana* disinfected of any infective eBSV sequences (Umber et al. 2016). More recently and in collaboration with the CARBAP, CIRAD has produced eBSV-free AAB triploid offspring (i.e. free from both infective and non-infective eBSV) in mimicking the conventional plantain breeding programme 4x/2x (Noumbissié et al. 2016). Finally, the molecular markers have been used to genotype all B-containing genotypes from the ITC germplasm collection pointing out that the biggest majority (>95%) of the accessions harbours at least one of the three infective eBSVs.

In this context, and through MusaNet, a small task force of specialists has devised a strategy to minimize the risks associated with the distribution of endogenous BSV-containing germplasm, while ensuring that recipients are fully aware of the potential issues. A position paper outlining this proposal (MusaNet 2015b) was drafted by the task force and circulated for public comment in July 2015 and presented to the GCDT in October 2015. This strategy will, by the end of 2016, open a large quantity of B genome material for distribution. In particular, a further 300 accessions conserved at the ITC would be available, raising the percentage of the ITC collection available for distribution from 60% to 90%.

SECTION 10.2 DISTRIBUTION AND SAFE EXCHANGE - WHERE WE WANT TO GO

A long-term goal of the *Musa* research community is that the effective conservation of maximum diversity will be permanently assured through a functional network of collections that actively contribute to and benefit from shared standards, strengthened technical capacity, and effective germplasm and information exchange. Through intensive characterization, collections will be rationalized and a core collection of accessions, embodying the total available *Musa* diversity, will be identified and held at the ITC and in 2-3 field collections. Plant health testing methods will be continually improved and updated, and the technology will be transferred to regional laboratories to maximize the health status of collections and facilitate free and safe exchange of germplasm.

10.2.1 Increased access to *Musa* germplasm

An important step towards increased accessibility is that contracting parties of the ITPGRFA indicate the collections that are under their control and in the public domain and make them available to all users through the use of a material transfer agreement. Crucial in this process is the provision of information on the legal frameworks and convincing stakeholders that the unrestricted flow of genetic resources is in the interest of all. In this respect, it would be very useful, through educational and public awareness programmes targeting the broadest range of stakeholders, to increase awareness of the importance of *Musa* genetic resources diversity and its access for conservation and use.

Assured access and distribution of *Musa* germplasm will result in benefits such as increased use of diversity in breeding programmes, increased knowledge of available traits, increased sharing of information, technologies and capacity building, reduced duplication of conservation efforts, and increased safety for the long-term preservation of material. If held only in national field collections or even *in vivo* in the wild, it could be under threat by pests and disease, or deforestation.

Access to wild and local diversity particularly should be further stimulated and incentives found, such as increased visibility for national collections for sharing the materials and duplicating them in the ITC.

It was recommended during the 2011 MusaNet meeting that more collaborative conservation initiatives between collections should be set up. For example, the Trust-supported project on conserving banana diversity for use in perpetuity aimed to increase the access and exchange of a wider diversity from a number of countries, through creating mutual benefits such as the upgrading of their collections, backing-up for health testing and cooperation in cryopreservation. Despite limited resources in many collections, around 150 accessions were duplicated at the ITC *in vitro* storage, and 132 accessions were cryopreserved at the ITC. New characterization data was entered into MGIS from several collections. The project also strengthened the virus-indexing capacity of the SPC in French Polynesia.

In general, better interaction with germplasm users and with the research community will help collections ensure that quality material is provided, thereby enhancing the exchange of germplasm.

It is also recommended that more functional partnerships are established for increased exchange, for better characterized materials and improved evaluation in a range of environments. Considering the high number of users carrying out evaluation trials with ITC germplasm, a strategy could be set up to encourage feedback of research results from users in order to better capture these evaluation data in public databases like MGIS.

At the global level, access to germplasm and associated information will be increased through better links between the ITC and the data providers from national collections into MGIS, providing regular updates on availability of new germplasm or new information on ITC accessions. An on-line ordering tool has been developed in MGIS to facilitate selection of the most appropriate germplasm from the ITC and linking accessions to an automatically generated SMTA. The system should also integrate a feedback system for information on distribution and monitoring use of germplasm from partner collections. See Chapter 7 - *the ITC Global* Musa *Collection* and Chapter 9 on *Information Management* for more information.

As of 2015, over 800 accessions from the ITC have also been made available to users in the form of lyophilized leaf samples. Although leaf samples are only used for DNA studies (plants cannot be regenerated from leaves), the leaf bank is a cost effective way to preserve the molecular materials and representative information from each species and cultivar in the collection, serving as a future reference and facilitating the exchange of genetic materials among molecular scientists. Compared with living plant materials, distribution of dried leaf samples offers the advantages of less to no quarantine and SMTA concerns, lower transportation costs, minimal risk of deterioration in transit and significantly reduced delivery time.

The revised *Technical Guidelines for Safe Movement of* Musa *Germplasm* (Thomas 2015) brings up to date information concerning virus indexing to determine the phytosanitary status of the germplasm for research, conservation and basic plant breeding purposes (including plant biotechnology). It is also important that the field collections have access to the necessary indexing expertise in regions to minimize the risk of transferring virus-infected material. Where necessary, training in approved indexing methodologies should be given. An accreditation system for indexing laboratories would allow more confidence in the regional movement of germplasm. A ring test developed via the Regeneration project could be extended to more collections having the facilities and skills to perform virus indexing using ELISA and PCR methods. This could serve as a pre-indexing and thus improve overall cost efficiency.

10.2.2 BSV issue and safe exchange

BSVs are a major impediment to the international transfer of valuable germplasm. Determination of which B genome accessions are at risk of endogenous BSV activation is a priority, as this knowledge would assist in the selection of infected cultivars for virus elimination and potential distribution. There was an informed discussion on the relative risks and advantages of distributing this material, and a consensus was reached among stakeholders following the publication of the MusaNet position paper in October 2015 (www.musanet.org). The agreed proposal includes a relaxation of the rules by fully informing the recipients of the risks of endogenous BSV and allowing the importing country to make the ultimate decision on receiving germplasm which contains infective endogenous BSV which may be activated at some stage. This will open up hundreds of accessions to the research community as well as allow the ITC to achieve its performance goal of making 90% of the collection available for distribution.

Also concerning the BSV problem, continued research into viruses and virus therapy is required. The main needs are to shorten virus indexing time, keep indexing protocols up to date and efficiently manage BSV-infected accessions.

One of the main recommendations to facilitate the exchange of germplasm is to focus on what scientists can do to ensure that research results are published and publicly available. The spirit of the ITPGRFA is to benefit all and not just one or few countries. A network such as MusaNet can help in facilitating partnerships for increased exchange of well-characterized material and improved evaluation in a range of environments. The incentives for national collections are the assurance that their collections are safely backed up and documented by sharing their materials with the ITC and to also putting more focus on the value and use of their collections' diversity.

There is a need for more exchange of disease-free material between countries within regions. Working toward this goal, regional networks will work to strengthen their virus-indexing capacity, by building up *in vitro* collections, optimizing plant conservation and multiplication strategies or equipping collections with virus-indexing kits customized to detect predominant diseases within the region (e.g. banana bunchy top virus). Such mechanisms should be developed in consultation with national authorities, regional agricultural research-and-development fora, and relevant organizations and statutory bodies, such as the International Plant Protection Convention (IPPC) with the secretariat at FAO and regional and national plant protection organizations.

10.2.3 Recommendations

Given the various users' feedback outlined in this section, the recommendations of the Global Strategy concerning distribution and safe exchange are to:

- Increase the distribution of germplasm (B genome) with infectious endogenous BSV
- Clarify the legal status of germplasm in some national collections
- Help in overcoming legal and practical barriers to free exchange by consulting with stakeholders on critical issues (e.g. phytopathologists, curators, and plant protection organisations)
- Increase the involvement of farmers, seed systems and multiplication centres in consultation processes
- Increase public awareness of the importance of *Musa* genetic resource diversity at the global level but also at local level via involving schools (the leaders of tomorrow)
- Ensure quality control mechanisms support national collections in being fully and safely accessible by establishing Quality Management Systems (QMS) in genebanks (See Chapter 7 on the ITC)
- Better identify the users and their needs through follow up surveys sent by genebanks (eg ITC) to recipients of germplasm
- Focus on the needs of breeders and phytopathologists through increased consultation and collaboration among breeders, researchers, national collections and genebanks.

SECTION 10.3 – DISTRIBUTION AND SAFE EXCHANGE - HOW WE WILL GET THERE

The ultimate aim of the global system for *ex situ* conservation is to conserve the entire *Musa* genepool in perpetuity and promote the safe exchange of a wide range of diverse *Musa* germplasm. The distribution and safe exchange work plan is as follows:

Factors	Major factor
1. Increased awareness of the need for high health status germplasm	• Update and extend the disease Factsheets (http://www.promusa.org/Pests+and+diseases+portal) following the recently updated the *Technical Guidelines for Safe Movement of* Musa *Germplasm* (Thomas 2015)
2. Continue to improve the efficiency of virus indexing protocols	• Review current indexing protocols to highlight deficiencies and inefficiencies • Assess potentially useful alternative virus-indexing methods and adopt those found to be superior
3. Increase the capacity for virus indexing in national and regional centres	• Survey to determine links between collections and diagnostic facilities, and the resources and expertise available at these facilities • Determine key laboratories in a position to assist strategically important collections • Extend the use of a ring test to more collections having the facilities and skills to perform virus indexing using ELISA and PCR methods. • Prioritize training and resource needs of diagnostic laboratories • Prepare a standardized virus indexing manual consistent with the updated *Technical Guidelines for Safe Movement of* Musa *Germplasm* • Provide training for diagnostic laboratories
4. Increasing of the capacity for producing clean materials for exchange	• Identify key laboratories with expertise in tissue culture to act as local multiplication centres • Build capacity for improved production practices, i.e. use of tissue culture, screenhouse multiplication

CHAPTER 11.
EVALUATION OF *MUSA* GERMPLASM

SECTION 11.1 EVALUATION - WHERE WE ARE NOW

11.1.1 Introduction

According to the FAO 2nd State of the World Report on PGRFA (FAO 2010), one of the most significant constraints to the use of plant genetic resources is the lack of publicly available evaluation data for most accessions -even on standard agronomic and physiological traits- and the capacity to manage such data. Breeders and other researchers require readily available and comprehensive datasets to select germplasm for further studies, for use in breeding programs or for testing and promotion to farmers and other end-users. Data on agronomic traits, host reaction to pests and diseases and abiotic constraints, post-harvest characteristics and quality (and an integration of the available knowledge) are crucial to help scientists and potential end-users select the right materials.

There are different stages and levels of germplasm evaluation, including:

1. *In situ* assessment of genetic resources and compilation of indigenous traditional knowledge, to guide early selection and acquisition of new accessions with relevant traits into collections
2. Preliminary evaluation of accessions in *ex situ* collections, recording relevant observations on basic traits, such as general performance in the specific environment or fruit quality
3. Targeted screening of a wide range of germplasm for specific traits of interest through phenotyping and genotyping, including high-throughput mass-screening under controlled conditions
4. Evaluation in early stages of selection and preliminary yield trials to assess the performance of accessions for specific traits under field conditions
5. Advanced yield trials in multiple locations to fully assess the influence of the environment and growing conditions on the performance of promising accessions
6. Farmers' participatory trials in target end-user environments to select end-user-preferred accessions for cultivar release and wide-scale adoption.

These stages are not always clearly delineated, nor do they always take place in a linear (sequential) way.

11.1.2 Current status of *Musa* germplasm evaluation

It is estimated that there could be as many as 1,000 different banana cultivars (INIBAP 2006) and possibly up to 70 wild species (Häkkinen and Väre 2008). According to the MusaNet survey of *Musa* collections, 56 institutes conserve over 15,000 accessions (see Table D.2 - *Institutes managing* Musa *genetic diversity* in Annex D) that are thus potentially available for use by breeders, researchers, farmers or consumers. Below, we attempt to give an assessment of past and current evaluation activities of this banana diversity, and the main lessons learnt.

11.1.1.1 In situ *characterization and documentation of indigenous traditional knowledge*

Besides information on the taxonomic status of collected genetic resources and general information on the collection site, the Descriptors for Banana (*Musa* spp) (IPGRI-INIBAP, CIRAD 1996) also recommends collecting information on the use of the fruit and other plant parts, and on environmental conditions at the collection site. These can give a first indication of the value of the material and its host reaction to the prevailing biotic and/or abiotic constraints. The local name(s) given to banana genetic resources also often

reveal useful information about the characteristics of the material. In addition, the so-called collector's notes, which capture relevant observations by the collector or local knowledge on traits of interest, are particularly informative and show that there is a wealth of knowledge to be captured at the time of collection.

11.1.1.2 Evaluation activities carried out by Musa ex situ *collections*

In the Global *Musa* Survey, between 48 and 50 collections provided information on the characterization, evaluation and breeding activities carried out with the germplasm from their collections.

Table 11.1. *Characterization, Evaluation and Breeding activities carried out by* ex situ *collections.*

Activity	Carried out on a routine basis	Carried out occasionally	Not carried out
Characterization for taxonomic traits (flower, fruits, etc.)	58%	38%	4%
Characterization using molecular markers	14%	36%	50%
Evaluation of host reaction to pests and diseases	35%	44%	21%
Evaluation of other important traits	50%	38%	12%
Breeding (hybrid or clonal trials)	26%	23%	51%

Half (50%) of the collections regularly carry out evaluation of other important traits, in addition to host reaction to pests and diseases (35%), and a quarter (26%) carry out hybrid or clonal trials on a routine basis. See Table 11.1 above for detailed results.

It thus seems that a significant number of accessions in collections are still not evaluated. In addition, for those accessions that have been evaluated, it is often hard to get access to the data. For example, only about one fourth of the 3,630 accessions recorded in MGIS have evaluation data, and only for a limited set of agronomic traits. These data most likely underestimate the real percentage of accessions evaluated and many more data for a range of traits may exist that are not necessarily available through MGIS.

11.1.1.3 Screening for agronomic performance and resistance to biotic and abiotic stresses

A quick review of publications in Musalit (www.musalit.org) dealing with germplasm evaluation indicate that most of these report on agronomic performance or host reaction to pests and diseases. Data on response to abiotic constraints and fruit quality traits appear to be less available. This is not surprising given that the principal traits sought by plant breeders are related to yield and its components (FAO 2010), and pest and disease resistance. We see the same trend in banana, with formal cultivar selection and crop improvement programs mostly focusing on a limited number of economically important traits, such as yield or disease resistance.

Despite this focus, there are still a number of important diseases for which no or very few natural sources of resistance have been identified. For instance, alarmingly few sources of resistance have been identified to Fusarium wilt tropical race 4 (TR4), which is already a major constraint to banana production in Asia and is spreading to other parts of the world. TR4 could potentially affect the lives of hundreds of millions of people dependent on bananas if no resistant cultivars with good consumer acceptance and market potential are available and adopted.

Drought is another serious constraint to banana production, for which there is little knowledge about tolerant cultivars. Some progress has been made in recent years to investigate the mechanisms underpinning drought tolerance, but more systematic, reliable field studies to screen the genepool for tolerance to drought is needed. With drought incidence expected to worsen in the near future, there is an urgent need to identify cultivars that are appropriate for drought-prone areas.

11.1.1.4 The International Musa *Testing Programme (IMTP)*

The International *Musa* Testing Programme (IMTP - http://www.promusa.org/IMTP) was established in 1989 by INIBAP in response to the needs of national programmes to provide famers with banana cultivars resistant to the major diseases affecting production. It was set up as a collaborative effort coordinated by Bioversity International to evaluate elite banana cultivars in multiple sites worldwide, using agreed evaluation protocols (Carlier et al. 2002; 2003).

There have been three phases of IMTP (starting in 1989, 1996 and 2005). In phase I, seven tetraploid hybrids developed by the banana breeding programme of FHIA in Honduras were tested for resistance to black leaf streak in six countries. Four years later, the recommendation was made to release three clones for distribution (Jones and Tézenas du Montcel 1994): FHIA-01 and FHIA-02, both dessert banana cultivars with outstanding agronomic performance and high resistance to black leaf streak, and FHIA-03, a cooking banana that also performed well. These three clones have since been distributed to more than 50 countries worldwide. In phase II, four programmes (FHIA in Honduras; EMBRAPA in Brazil; the Instituto de Investigaciones en Viandas Tropicales (INIVIT) in Cuba; and the Taiwan Banana Research Institute (TBRI) in Taiwan contributed germplasm, and the number of testing sites increased from 6 to 37. The results (Orjeda 2000) suggested that, among the different materials tested, FHIA-23 and SH-3436-9 were the most tolerant to black leaf streak. The improved hybrid with the best overall performance was FHIA-23. An improved cultivar that deserves special mention is GCTCV-119, which had the lowest discoloration score for both *Foc* races and good yields under good management.

Eighteen countries participated in phase III to which five breeding programmes contributed germplasm (FHIA in Honduras; IITA in Uganda and Nigeria; TBRI in Taiwan; CARBAP in Cameroon; and the CIRAD in Guadeloupe). For the first time, some partners carried out in-depth studies that involved epidemiological and ecological research, while the others undertook simplified performance trials against specific diseases. A standard procedure for data management and statistical analysis was also developed. ‘FHIA-25’, an AABB cooking type, had the highest average annual yield, followed by ‘FHIA-17’, an AAAA Gros Michel type dessert banana (Crichton and Van den Bergh 2016).

The IMTP allowed for new materials coming out of breeding and selection programmes to be evaluated in a range of environments and to become more widely known to next users. The IMTP also stimulated further local testing of a subset of new materials with beneficial traits for the specific country or location. The materials included in the different phases of the IMTP have all been shared with the ITC and are available for distribution to interested parties. Trial sites, mainly geared to assessing resistance to Fusarium wilt, black leaf streak and later also nematodes, were increasingly also used for other evaluations (e.g. fruit micronutrient content) or to answer key questions about pathogen, disease and host interactions. The IMTP also sought to strengthen the capacity of national institutes to evaluate improved materials and to carry out research on banana for local consumption.

11.1.1.5 Participatory varietal selection

Adoption and impact on local communities of new banana cultivars has often been slow and lower than expected. This may be at least partly explained by the fact that evaluation programs often focus on a few economically important traits and that farmers are mostly involved only at the very end of the testing pipeline. This approach fails to take into account other potentially important traits for which the economic value may be more difficult to assess, such as traits related to consumers’ taste preferences, local recipes, cultural values, or the relative role of men and women in production, processing and marketing. Working in close collaboration with farmers during the whole evaluation process allows the quantification of the suitability of each cultivar to local farming conditions, while sensory evaluations with consumers provide feedback on taste and other organoleptic features, as well as processing potential.

While having shown its value in the evaluation and adoption of other crops, such as rice and potato, true participatory varietal selection is still relatively unexplored in banana. Only a handful of publications in Musalit specifically mention a participatory evaluation approach in their title, though it is believed that more participatory trials are actually being conducted.

SECTION 11.2 EVALUATION - WHERE WE WANT TO GO

11.2.1 Banana types and uses around the world

For cultivars to be used and adopted, they need to respond to the real needs and preferences of the target end-users, and this needs to be taken into account throughout the evaluation process. As highlighted above, evaluation programs often focus on a limited number of economically important traits, thus failing to take into account other potentially important traits for which the economic value may be more difficult to assess, such as traits related to consumers preferences (taste, flavour, processing ability, satiety feeling), cultural values, or the relative role of men and women in production, processing and marketing.

Banana cultivars, and the traits they are selected for, differ significantly between regions, and between locations within regions. Globally, different types of cultivars are grown for a variety of uses, and pest and disease constraints have different importance depending on the region, as environmental conditions are often complex and highly variable.

In Asia and the Pacific, the most important banana group is the Cavendish (AAA) type accounting for 59% of regional production, either for export (China, Taiwan, India, and Philippines) or for local use (Indonesia, Philippines, Vietnam, Cambodia, Australia and Thailand). Gros Michel (AAA) and other dessert bananas make up a further 16% of production (Philippines, Indonesia, India and Malaysia). The remaining 25% of production are cooking bananas, that are of medium to high importance in some countries, comprising AAB 'Maoli-Popo'ulu and Iholena', ABB Pisang Awak, Bluggoe, and Saba.

In West and Central Africa, plantains (AAB) are of major importance and account for 69% of the production, mainly for local consumption. Dessert banana (e.g. Cavendish, Gros Michel) are of medium importance (24% of production), both for export and local markets. In East and Southern Africa, highland bananas (AAA) and other cooking types (ABB) make up 76% of the total production and are of major importance for different uses. Plantains are of lower significance (7% of production) than in West and Central Africa and dessert types account for 17% of production in the region.

The bulk of production in Latin America and the Caribbean is of the Cavendish type, followed by plantains, other dessert types and other cooking types. The region is a major exporter of banana, accounting for 66% of global Cavendish exports, and also produces 72% of plantains traded on international markets. Nevertheless, 62% of banana production in the region is consumed locally, which indicates the high importance of the crop in local diets and food security throughout the region. Important cultivar groups for local consumption are Prata (AAB) in Brazil, Silk (AAB) in all Latin American countries, Gros Michel (AAA) in Central and South America in intercropping with forest trees, coffee and cacao, Bluggoe and Pisang Awak (ABB) in Nicaragua, Mexico and Cuba, and finally the important group of the Plantains (AAB).

If adoption of cultivars by end-users is the final goal, the trait preferences of the different stakeholders along the value chain -including producers, processors, traders, consumers- need to be taken into account throughout the evaluation process.

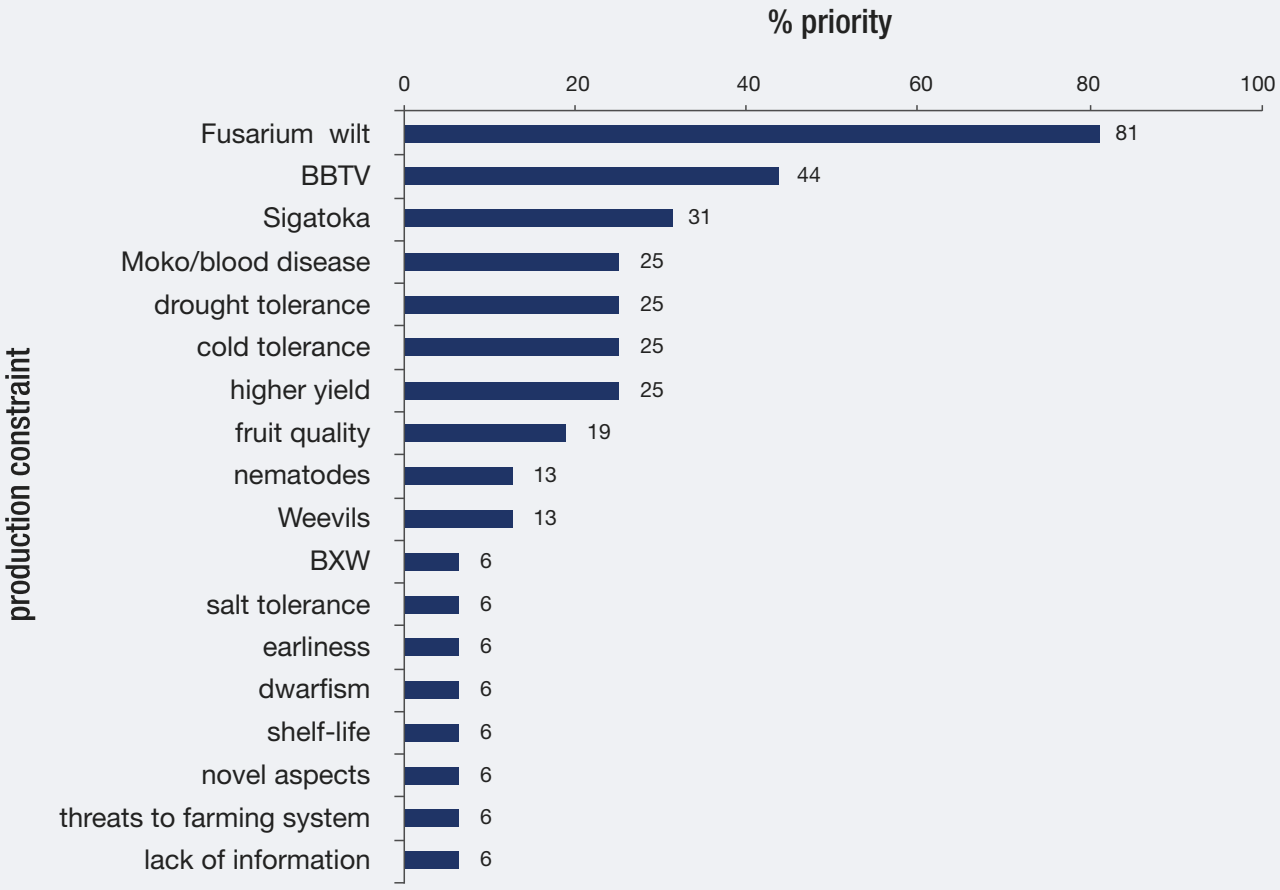
11.2.2 Traits to be evaluated

11.2.2.1 Global scale

At the MusaNet meeting in March 2011, the following traits were identified by the Evaluation Thematic Group (ETG) as being of global importance: resistance to Fusarium wilt, banana bunchy top and black leaf streak, drought tolerance, shelf life, dry matter content, vitamins and minerals content, height, yield and finger drop resistance.

In 2012, participants to the MusaNet/Trust meeting in Bogor, Indonesia confirmed the importance of the three major diseases previously identified, namely Fusarium wilt, BBTV and black leaf streak, and the main abiotic constraints, namely drought (Figure 11.1 below).

Figure 11.1. *Priorities assigned to the major production constraints by the participants of the MusaNet/Trust Bogor meeting in 2012.*



They also discussed the expected impact of climate change on *Musa* breeding objectives, and the need to evaluate more materials for more traits that could be of economic importance in the future. It was suggested that breeding programmes should focus efforts on the following traits in preparation for climate change: wind tolerance (shorter plants and strong root system for a better anchorage), drought tolerance (also including shorter cycles/early fruiting to avoid dry periods), salt tolerance, and plasticity regarding variation in rainfalls and succession of drought/flooding.

The 2013 RTB online research priority setting survey[1] of *Musa* experts eliciting the most important constraints to banana yields and farmers' income also saw that main production constraints varied significantly from region to region (see Table 11.2), but in all regions, and across the CGIAR, banana pests and diseases ranked highest, although there was little significant difference between most of these. They also varied according to cultivar group. In terms of diseases, black leaf streak[2] (BLS, or black Sigatoka) *Mycosphaerella fijiensis* ranked the most serious constraint, followed by Fusarium wilt[3] caused by the soil-borne fungus *Fusarium oxysporum* f. sp. cubense (Foc), then banana bunchy top virus[4] (BBTV), and banana *Xanthomonas* (bacterial) wilt[5] (BXW). The two pests, banana weevil[6] (*Cosmopolites sordidus* (Germar)) and burrowing nematode[7] (*Radopholus similis*) are considered slightly more threatening than BXW or BBTV.

11.2.2.2 Regional scale

Other traits are more of regional importance. Meetings held by the four banana networks (BAPNET, BARNESA, MUSALAC, Innovate Plantain[8]) are critical in identifying regional priorities. The networks are represented in MusaNet and thus contribute to MusaNet's objectives and activities. See Annex A on networks and partnerships for more information.

The most recent BAPNET consultation (2014) brought to the fore the need for the collection of new varieties and evaluation of accessions already held in regional genebanks for traits such as drought tolerance and disease resistance. Another priority is establishing a framework for investigating what diversity is being used in the region so that breeders as well as farmers can easily access what is already available.

MUSALAC, which meets every couple of years, has recently focused on Fusarium wilt tolerance, soil health and suppressiveness and climate change and adaptation. Screening Asian cultivars for TR4 tolerance is a current concern. They also proposed that information resulting from evaluation trials needs to be made available to the public through a searchable database in order to facilitate priority setting.

BARNESA priorities have included investigation into disease resistance, particularly Banana *Xanthomonas* wilt (BXW) and work on *in situ* and on farm conservation practices.

Innovate Plantain priority activities have focused on better management of pests and diseases, access to clean planting material and discovery and use of traits to mitigate the effects of climatic change.

During the initial MusaNet meeting and subsequent follow-up interactions, the ETG identified, for different types of bananas in different regions, the major traits that need evaluation (see Table 11.2).

Table 11.2. *Important traits for different banana types in different regions.*

1 http://www.promusa.org/tiki-read_article.php?articleId=92

2 http://www.promusa.org/Black+leaf+streak

3 http://www.promusa.org/Fusarium+wilt

4 http://www.promusa.org/Bunchy+top

5 http://www.promusa.org/Xanthomonas+wilt

6 http://entnemdept.ufl.edu/creatures/fruit/borers/banana_root_borer.htm

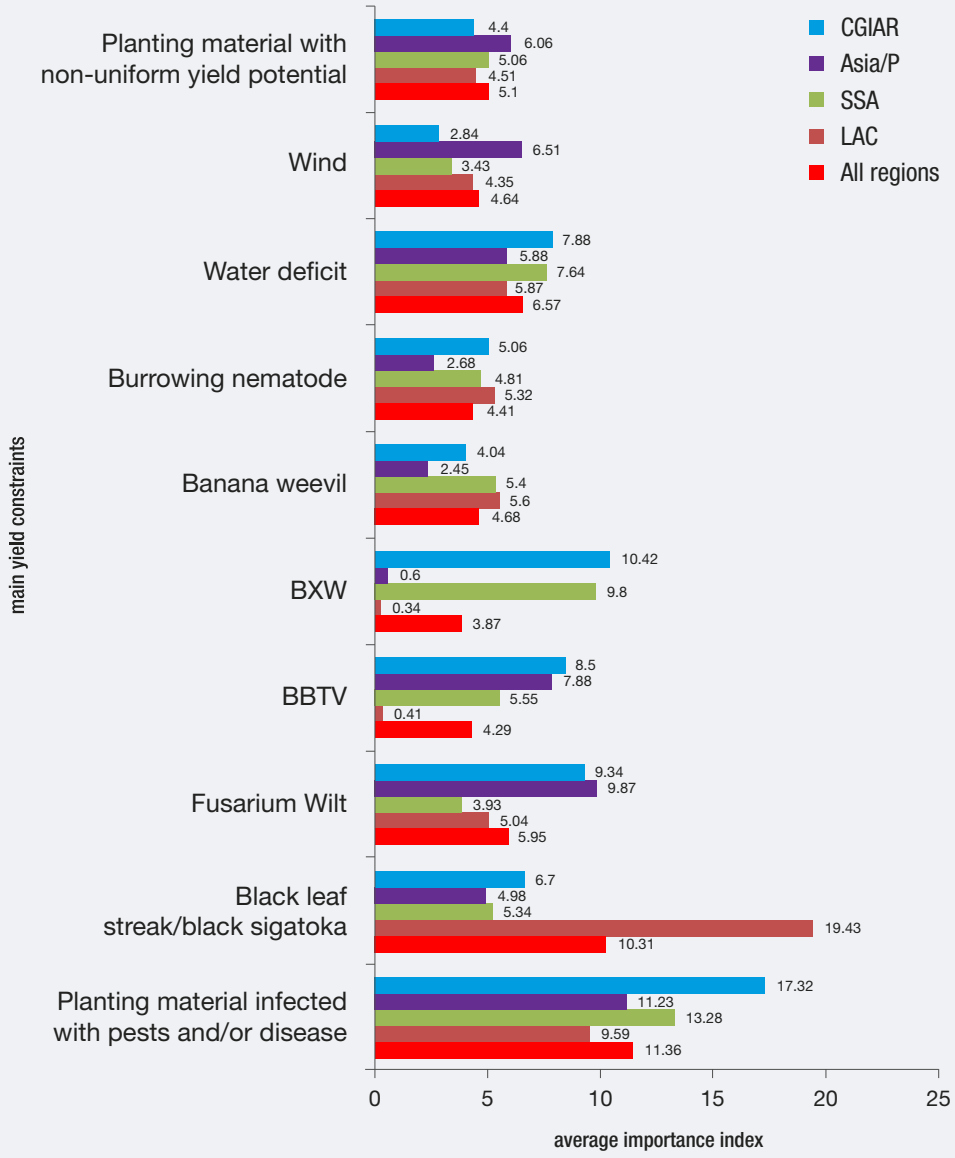
7 http://www.promusa.org/Radopholus+similis

8 http://banana-networks.org/

Banana Types	Asia-Pacific	West and Central Africa	South East Asia	Latin America and Caribbean
Cavendish export	Fusarium, nematodes	Yield, ratio bunch/box, plant height, crop cycle, BLS, YS, Fusarium R1, Tropical Race 4 (TR4), nematodes, weevils, finger drop, fruit taste, fruit green life, fruit tolerance to cold in post-harvest chain, peel thickness, peel splitting		Yield, ratio bunch/box, plant height, crop cycle, BLS, YS, Fusarium R1, TR4, nematodes, weevils, finger drop, fruit taste, fruit green life, fruit tolerance to cold in post-harvest chain, peel thickness, peel splitting
Cavendish local use	Fusarium TR4, BBTV, BLS, nematodes, drought, wind (typhoons)			
Gros Michel and other dessert	Fusarium R1, TR4, BBTV, BLS, nematodes, drought, wind (typhoons)		Bacterial wilt, Fusarium, BBTV	
Maoli-Popo'ulu and Iholena plantains	BLS, BBTV, nematodes, drought, wind (typhoons), vitamin A content	Nematodes, weevils, drought, Fusarium, BBTV, BLS, cooking quality (starch/fibre), nutritional compounds, finger drop, green/shelf life	Weevils, root system, BBTV	Cooking ability (starch), green/shelf life, fruit taste, nutritional compounds
East African Highland Bananas			Weevils, bacterial wilt, nematodes, drought, BBTV, BLS, Fusarium, Green/shelf life	Cooking ability (starch), fruit taste
ABB cooking bananas	Bacterial diseases			Cooking ability (starch), soluble solids

The 2013 RTB online research priority setting survey also saw that main production constraints varied significantly from region to region (see Figure 11.2 below). They also varied according to cultivar group. BLS is hugely important in Latin America); while Fusarium wilt is more important in the Asia Pacific region (although the virulent tropical TR4 has now also entered Africa). BBTV is much less important in Latin America than elsewhere, and BXW is much more important in Africa than the other two regions. The two pests, Banana Weevil (*Cosmopolites sordidus* (Germar)) and burrowing nematode (*Radopholus similis*) are more or less equally important in the different regions.

Figure 11.2. *Main production constraints per region, as identified by the 2013 RTB online research priority setting survey.*



One recent iniative that will contribute to a better understanding of regional diversity is the inventory of the top 10 *Musa* varieties in each country. This is being carried out in several regions: in Latin America and Caribbean, collections are being surveyed in 2016 about their usage of ITC material and their most successful cultivars, while in West and Central Africa, a discussion on the top varieties took place at the regional MusaNet workshop in Cameroon in 2015. One of MusaNet's objectives is to expand this inventory to all regions and make the results available to collection curators and breeders, so that they can select and test varieties that have been successful in other countries/regions.

11.2.2.3 Local scale

However, for effective adoption to take place, evaluation programs will also need to take into account end-user preferences at a much more local scale. Breeders and other researchers require information on end-user needs and preferences, and need to link these to traits that can be objectively measured.

SECTION 11.3 – EVALUATION - HOW WE WILL GET THERE

11.3.1 MusaNet Evaluation Thematic Group (ETG)

The overall goal of the MusaNet ETG is to enhance the value of *Musa* genetic resources for different end users (including farmers, curators, breeders, and researchers) and thus encourage their use to improve the sustainability of banana productions systems and farmers' livelihoods.

Musa genetic resources (including wild species, landraces, cultivar selections and improved materials) need to be evaluated for traits of interest, such as agronomic performance, host reaction to pests and diseases and post-harvest characteristics, under diverse environmental conditions through a user-oriented network. This process is expected to result in a better understanding of the *Musa* genepool and of the interactions between *Musa*, its major pathogens and the environment, and will eventually lead to an increased efficiency of banana breeding programmes and the development of more robust farmer recommendations. The resulting information and knowledge needs to be compiled and made available, and important traits highlighted to potential users.

As part of a global network, the ETG will focus on traits of global relevance where cross-regional collaboration and learning is important and on traits of special importance in regions where high food insecurity and poverty incidence coincides with high importance of banana for food security or income generation. To still capture locally important traits, gender-sensitive participatory rural appraisal tools can be used to document the needs and preferences of the different actors along the banana production and value chains, to achieve a better understanding of underlying factors that determine the value that farmers and consumers attribute to different traits, and the criteria that they use for adoption or rejection of cultivars. Special attention should be given to the different roles, needs and preferences of men and women.

The three main user groups identified are: breeders, the research community and farmers. The members of the ETG will have to work in close collaboration with researchers from different disciplines (agronomy, plant pathology, post-harvest, crop physiology, etc.), members from the other MusaNet Thematic Groups, curators of collections and many others.

The four regional networks need to play an essential role in terms of regional coordination of activities, and ensuring the link with the end-users (farmers and consumers). They should continue to share their priorities with the ETG for future planning of activities within MusaNet. There also needs to be close collaboration with the ProMusa community, especially in the area of knowledge management.

In order to allow comparison of evaluation data for specific traits between different environments and over time, there is need to develop and agree on a set of standard "best-practice" protocols for priority traits, as well as a standard set of reference cultivars. The ETG can play a role in the development of such guidelines.

11.3.2 Global evaluation platform

Despite the positive results of the previous phases of the program, the IMTP has also received some critical feedback, with the most important one probably being the fact that the adoption of the new cultivars has in general remained low. In addition, a real analysis of the interaction between the performance of the different genotypes and the environment (GxE analysis) was often not possible. Also, the trials were expensive, and most trials were carried out at the partner's own expense.

Therefore, the format of future trials is changing to maximize chances for adoption of the new cultivars. Trial groups will be organized around certain banana types and targeted to relevant end-user environments. A variety of participatory rural appraisal tools will be used to identify, prioritize and document the needs and preferences of the different actors along the banana production and value chain.

Two types of trials will be run consecutively: on-station trials to gain accurate data on cultivar performance, and on-farm participatory selection trials to facilitate access to and testing of the new material by end users

(known as 'mother-baby' trials). The experimental design of the on-station trials has been optimized to include better characterization of the abiotic environment, simplification of the variables to be measured, and the grouping of variables into core and additional modules. Farmers are being invited to visit the on-station trials and select a subset of cultivars for testing in their own fields. The on-farm trials are fully managed by the farmers, who will rate the performance of the new cultivars in comparison with their local checks. Through farmers' group discussions, household-level surveys and stakeholders' interviews, and linking socio-cultural traits and taste preferences with morphological and physicochemical fruit properties, a better understanding will be achieved of underlying factors that determine how farmers and consumers make choices, the value they attribute to different traits in their local context, and the criteria they use for adoption or rejection of cultivars. Special attention will be given to the different roles, needs and preferences of men and women.

It is hoped that the new format will enable a more efficient mechanism for the evaluation of banana cultivars, and lead to faster adoption and greater impact on the lives of banana-dependent populations.

11.3.3 Strategic plan

The MusaNet ETG will help coordinate banana evaluation activities globally, by bringing together experts in the field, reviewing currently available information, standardizing evaluation protocols to allow comparison of performance between locations and over time, establishing a framework for data compilation and analysis, and communicating the results and available knowledge to relevant end-users.

More details on each of these activities are given in Table 11.3 below.

Table 11.3. *Proposed objectives and actions for* Musa *evaluation.*

Specific objectives	Actions
1. Comprehensive assessment of currently available evaluation data	• Review of literature on evaluation of *Musa* genetic resources • Review of currently available phenotypic and genotypic evaluation data information in MGIS and other collection databases • Identify major gaps in knowledge in terms of traits and accessions
2. Standardization of evaluation protocols	• Review currently available phenotyping/genotyping methodologies for evaluation of priority traits • Identify gaps in evaluation methodologies; identify for which traits and/or types of evaluation good protocols are not available • Develop and agree on a set of standard "best-practice" protocols for priority traits, and enter standardized traits/methods in Trait Ontology • Agree on a set of standard check genotypes for all trials • Identify a set of well characterized (climate, soil conditions, etc.) reference trial sites
3. Set up framework for data compilation and analysis	• Compile existing evaluation data in *The Global Agricultural Trial Repository of CCAFS* (AgTrials) (www.agtrials.org) • Ensure link between AgTrials and MGIS • Ensure link between AgTrials and Trait Ontology • Engage in global analyses for germplasm performance and GxE interactions
4. Information and knowledge sharing	• Make available and pro-actively share information and knowledge with the broader *Musa* research community and other users/stakeholders, in collaboration with MusaNet's Information Thematic Group and the global network ProMusa (www.promusa.org) • Make available a database search tool for information on different varieties that are being screened, such as agronomic, climatic and quality characteristics, in order to help priority setting in the regions.

CHAPTER 12.
GENETIC IMPROVEMENT

SECTION 12.1 GENETIC IMPROVEMENT – WHERE WE ARE NOW

Genetic improvement presents a potentially cost-effective mechanism to address current constraints in smallholder and commercial production by providing high-performing cultivars adaptable to diverse environments.

Productivity, resilience and sustainability may be enhanced by integrating inter- and/or intra-crop diversity within production systems. Experiences with rice and other cereals, being extended to banana, suggest that the risks of losses from epidemic diseases can be mitigated by planting mixed genotypes in place of extensive monocrops of a single cultivar. A demand for increased diversity of cultivars, as well as improved cultivars, exists among smallholder farmers and formal market systems, as well as within the research and breeding community. Supplying producers with a wider range of diversity can potentially enable more livelihood options to be adopted and family nutrition to be similarly diversified.

This chapter does not intend to review banana breeding. For exhaustive recent reviews, the reader can refer to Ortiz et al. 1995, Bakry et al. 2009, Tenkouano et al. 2011, Ortiz 2013, and Ortiz and Swennen 2013.

12.1.1 Breeding objectives

Banana breeding began in the early 1920s, when all the large commercial plantations of banana for export in Central America were decimated by the Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Cubense*. In response the first breeding program was initiated in Trinidad and Jamaica by the Imperial College of Tropical Agriculture (ICTA), paving the way for the implementation of several national programs some 40 years later. Meanwhile, the fungus spread around the world and reached all plantations of AAA Gros Michel, the then-predominant cultivar, so that banana export in the 1950s was at risk of disappearing. Fortunately, explorations of existing genetic resources showed the AAA 'Cavendish' cultivars to be resistant to the disease and suitable for export. These cultivars subsequently substituted 'Gros Michel' in all commercial export plantations worldwide and remain the predominant subgroup produced at the global level, both for export and domestic markets. Obviously, a production system based on such a narrow genetic base is extremely vulnerable to existing and emerging pests and diseases.

Until the 1970s, banana breeding strongly focused on export bananas, mainly led by ICTA and the United Fruit Company, who established the FHIA breeding program in Honduras. During the second half of the 20th century, several diseases and pests increasingly affected various banana types, thus menacing the production at the farm level as well: Sigatoka leaf diseases (yellow Sigatoka, followed by the more damaging black leaf streak), nematodes and weevil borers. More recently, the emergence and rapid spread of Fusarium wilt tropical race 4 in Asia, to which the Cavendish is susceptible, became an additional priority.

Consequently, from its initial focus on dessert export bananas, banana breeding expanded gradually to plantain and non-export dessert bananas in the 1970s, considering their importance as major staple foods and sources of income for millions of people in tropical and subtropical countries. National and international programs were established to address national, regional or global needs: IITA and CARBAP in Central and East-Africa, EMBRAPA in Brazil, CIRAD in the Caribbean, NRCB in India, etc. Despite the importance of bananas and plantains in food security, there are still only a small number of breeding programs. A major

step towards a solution for such a critical situation was the establishment in 1985 of the International Network for Improvement of Banana and Plantain (INIBAP), now Bioversity International, in order to coordinate and stimulate collaboration within the banana research community, to facilitate the exchange of genetic resources and knowledge and to foster international cooperation.

Improvement for pest and disease resistance or tolerance is the primary objective of banana breeding, but the simple release of new resistant cultivars is insufficient for actual adoption by producers. Hybrids must respond to a wide number of criteria according to their specific cropping and socio-economic contexts. For export banana, breeding aims at cultivars that maintain or improve the productivity and the fruit quality (including post-harvest and transportation and conservation qualities) of Cavendish clones. For local consumption and domestic markets, most criteria are the same, even if post-harvest criteria are less constraining. Secondary objectives are linked to the diversity of cultivar growing environments, and include tolerance to cold, to drought stresses, short plant size and strong root system to avoid wind damage and optimise nutritional uptake, etc.

As stated by Ortiz (2013), “emphasis for banana genetic enhancement should be given to pre-emptive breeding – particularly through broadening approaches – to deal with new strains of major pathogens and pests, as well as other emerging constraints”.

12.1.2 Biological constraints

Most cultivated bananas are triploid (2n=3x=33), originating mostly from the two diploid species *M. acuminata* and *M. balbisiana* (2n=2x=22). Banana breeding faces a paradox: considerable amount of seeds are required to produce large progenies, but hybrids selected from these populations must not contain any seeds.

Wild species display few reproductive barriers: the male flowers have plenty of viable pollen and the fruits are full of seeds. One thousand to over 10,000 seeds per bunch can be found in wild *acuminata* or *balbisiana* species. Actually, breeding progress is hampered by the specific biology of cultivated bananas, i.e. low reproductive fertility of cultivars. Fruit sterility and parthenocarpy have been selected by growers at the diploid level throughout the long domestication history of this crop. At the diploid level, infertility is partly associated with structural heterozygosity, present in most cultivars, leading to aneuploid gametes bearing from 12 to 16 chromosomes. Fertility is zero in AB diploid cultivars because of the partial homeology between the *acuminata* and *balbisiana* chromosomes, whereas AA diploid cultivars show a wide range of male or female fertility. Although still rather low, their overall fertility is often higher than triploids: occurrence of aneuploid gametes and formation of diploid to tetraploid gametes are frequently reported at the triploid level. This results in little or no fertility in triploid cultivars, mainly due to the uneven number of chromosomes.

Even a profusely hand-pollinated bunch of a triploid cultivar rarely contains more than 1 to 5 good seeds, making the production of a significant offspring a great challenge. Beyond scarcity, seeds in cultivated clones are often abnormal, indeed, with the absence of embryo or endosperm, sometimes both, and fail to develop into seedlings. Greenhouse germination rates are rarely over 20%, and most banana breeders systematically resort to embryo rescue using tissue culture to increase the germination rate up to 85%.

12.1.3 Breeding approaches

For most banana breeders, the main strategy for genetic improvement of banana is to breed resistant triploid hybrids as final products. Triploid cultivars were proved to give a selective advantage over other ploidy levels: diploid cultivars are usually less productive and less vigorous although some diploid clones, like AAcv Pisang Mas of ABcv Kunnan are of some significant value but produced for small or niche markets with high added value. Tetraploid hybrids were the first resistant cultivars bred, and may actually be satisfying in terms of yield, bunch and fruit sizes. However, tetraploids occasionally contain seeds and their poorer fruit quality has never met the requirements for large scale adoption by markets and consumers.

Basically, two breeding philosophies have been elaborated by banana breeders. The first one has been called “Evolutionary breeding” (Tenkouano et al. 2011) and relies on the improvement of the triploid cultivars crossed with diploid cultivars. Since the method is not reproducing the evolution towards triploids via edible diploids (see Chapters 2 and 3), this document prefers to adopt the name “Pragmatic breeding”. The second approach, “Reconstructive breeding”, is built on the use of diploid germplasm to create triploid hybrids, thus trying to mimic the spontaneous development of the current triploid cultivars from their diploid ancestors in the past.

12.1.3.1 The « Pragmatic breeding » approach

The origin of this approach is at the dawn of banana breeding, when breeders tried to develop ‘Gros Michel’ hybrids resistant to Fusarium wilt. Triploid cultivars show a residual fertility and when crossed with diploid cultivars, may produce a few seeds. The primary products issued from these crosses show a considerable genotypic (and phenotypic) variation, ranging from diploid to highly polyploid hybrids. Among them, tetraploid hybrids, arising from unreduced triploid egg cells (2n=3x=33) were selected. The value of these hybrids is that the genes from the mother plant do not segregate and their main characteristics are maintained in the tetraploid, while the haploid genome added from the diploid confers disease resistance.

This strategy was taken up for other dessert and cooking bananas to confer resistance to black leaf streak and to nematodes. Dessert tetraploid hybrids were developed at FHIA from crosses between dwarf mutants of Gros Michel and Prata with improved diploids resistant to Sigatoka diseases and nematodes. Cooking bananas were developed from crosses between plantains and resistant diploids at IITA, CARBAP and FHIA to confer resistance to black leaf streak. Some outstanding hybrids were obtained by this strategy. FHIA21, a cooking tetraploid hybrid released by FHIA, is now being cultivated for local markets in some countries in West Africa, in Central and South America and in the Caribbean, as a substitute to black leaf streak susceptible plantains. A major outbreak of this approach is the discovery of endogenous integrated sequences of the endogenous banana streak virus (eBSV) in the plantain genome, releasing infectious viral particle in the progenies issued from crosses. However, these viral sequences have been shown to behave as pseudo-genes and can in some cases segregate as heterozygous locus (Gayral et al. 2008), paving the way for the elimination of infectious eBSV sequences.

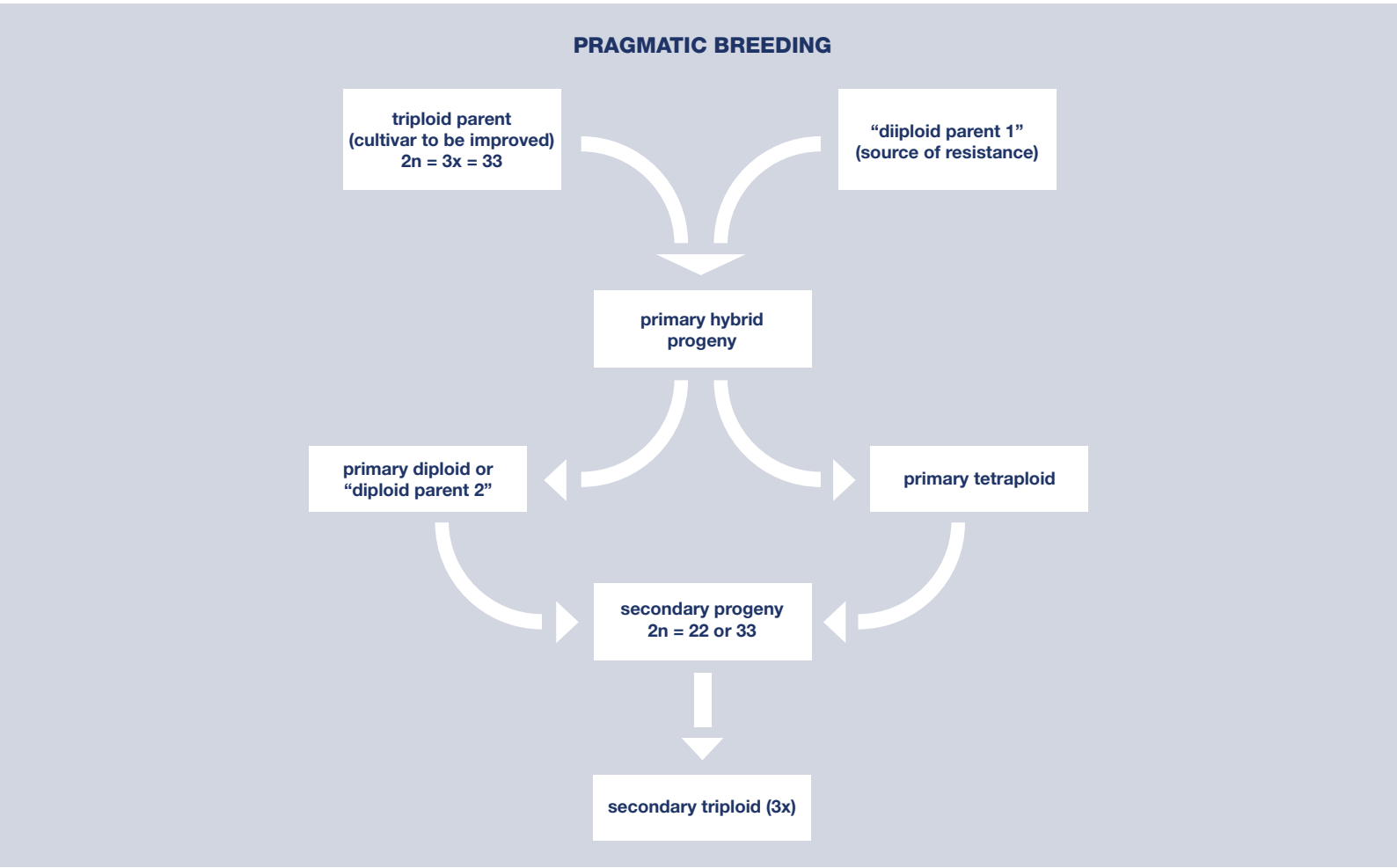
Tetraploid hybrids, AAAB, AAAA and AABB (primary tetraploid hybrids) are often much more fertile than the triploid parent, and can produce seeds when crossed with a diploid accession. The descendants obtained from these 4x x 2x crosses are predominantly triploids (secondary triploid hybrids). Thanks to redistributions and recombinations between A and B chromosome during meiosis, triploid hybrids free from infectious eBSV can be obtained from theses crosses.

As mentioned above, the initial 3x x 2x cross also produces diploid hybrids, whose genetic background comes from the triploid mother plant. These primary diploid hybrids are eventually used in the 4x x 2x cross to bring additional mother-plant background to the secondary triploid hybrids.

In this breeding scheme, selected resistant primary tetraploid hybrids can be either released as new improved cultivars or subsequently crossed with other improved diploid selections to obtain secondary triploid hybrids. In this approach, the genetic diversity used on the triploid side is very limited, due to the low fertility of triploid cultivars. The choice of the diploid parents to be crossed in the first or second step is therefore crucial. Considering that the unreduced triploid eggs of the maternal parent are genetically homogenous, the breeding effort is only based on the improvement of the diploid parent. Within the great diversity of the diploid pool, wild and edible diploids have been selected to introgress pest and disease resistance in triploid cultivars. However, wild relatives are highly fertile but have very few of the outward appearances of cultivated bananas, while edible diploids, even if they are more attractive in terms of bunch appearance and fruit qualities, are at most moderately fertile and often not resistant to diseases. Consequently, pre-breeding at the diploid level appears as a preliminary step. Some elite diploids have

been developed and used, the most notables are the AA hybrid M53 (resistant to Sigatoka diseases and to Fusarium wilt), bred in the 1950s by the breeding programme of the former Jamaican Banana Board, and several outstanding diploids created at FHIA, some of which have multiple resistance (to Sigatoka diseases, nematodes and Fusarium wilt) and a huge number of hands.

Figure 12.1. *Pragmatic Breeding (Source: J. P. Horry)*



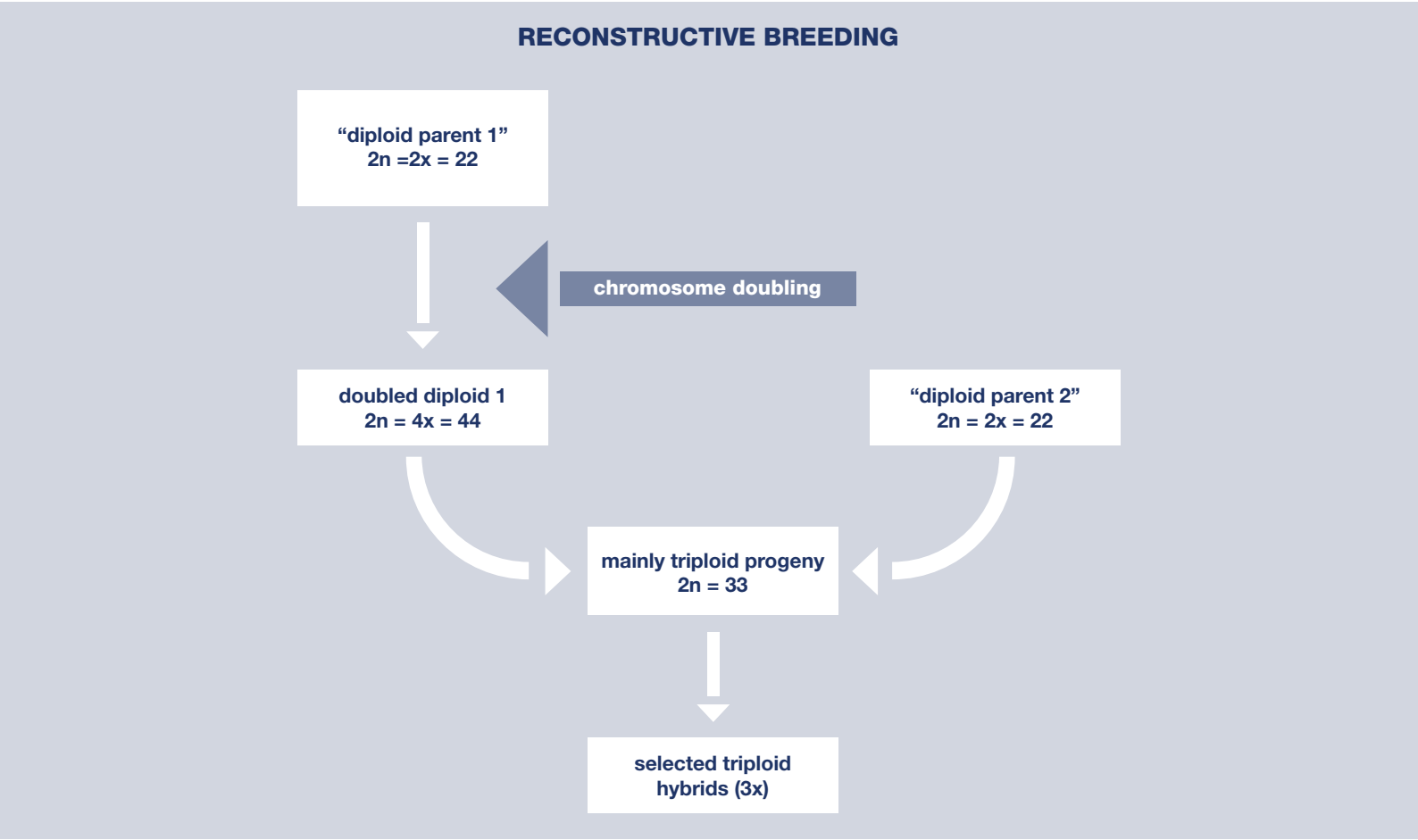
12.1.3.2 The « Reconstructive breeding » approach

Several years ago, an original breeding approach was developed at CIRAD relying on the use of the diploid genetic stocks to create triploid hybrids. Its rationale was corroborated by the recent discovery from genomic studies of the most likely ancestors of the popular triploid cultivars 'Gros Michel' and the Cavendish subgroup. This now guides the precise choice of the most relevant parents (the putative ancestors or their close derivatives) to create *de novo* improved variants of the triploid cultivars.

The breeding scheme is also based on a search of the best specific combining abilities between two diploids of which one is the donor of diplo-gametes. Based on complementarities, this approach aims to associate the favourable traits brought by both parents and to maximize the heterozygosity in the triploid progenies. However, since the production of 2n gametes is uncontrolled and fairly rare in diploid clones, a more regular production of 2n gametes is achieved by the use of colchicine to induce tetraploidisation of one of the diploid parents prior to crossing with the other diploid parent, donor of the complementary n-gamete.

Diploid candidates, wild or edible, are selected according to the type of banana to be developed (cooking or dessert), their agronomic and fruit quality characteristics, their behaviour with respect to diseases and their paternal and/or maternal fertility, and their parentage with triploid cultivars. New diploid hybrids that express high resistance to various diseases can also be included in the breeding scheme. Following a primary phase evaluating pest and diseases resistance, agronomic and quality features and fertility, diploids are selected and treated *in vitro* with colchicine for chromosome doubling to form auto-or allotetraploids, whether they are AA or AB cultivars.

Figure 12.2. *Reconstructive Breeding (Source: J. P. Horry)*



The ability to set progeny is strongly linked to gamete fertility, which is highly variable from one to another clone. The gamete fertility of the doubled AA diploids is rather unpredictable: some clones are fertile at both diploid and tetraploid levels. Others are completely sterile at the tetraploid level. Conversely, all interspecific AB clones studied are sterile at the diploid level but have been shown to be systematically male and female fertile at the tetraploid level. These last results confirm previous studies of cytogenetics stating that gamete sterility in AB clones is probably due to incomplete chromosome pairing at meiosis.

An intensive programme of hybridization combining various parents and a larger number of doubled genotypes have strongly indicated that the studied doubled-diploid clones are almost exclusively producing polyploid gametes containing, for a major part, exactly 22 chromosomes. Then, the progenies obtained from doubled-diploids x diploids are essentially triploid, which is the expected objective. These first outcomes validate the original pathways of banana improvement developed by CIRAD.

The advantages of this approach are manifold: the genetic combinations selected at the diploid level are totally or partially preserved through the doubles-diploid. Triploidy confers a high level of sterility. Moreover, breeders can use the wide diversity of the diploid genetic pool, including the very fertile wild parents that enable production of large triploid progenies in which it is easier to make an effective selection. Theoretically, it allows the introduction of new selection criteria at any stage (by using new parents) to respond quickly either to the appearance of new races of pathogenic fungi or to meet other selection objectives.

12.1.4 The Current Major Breeding Programmes

This section is intended to provide a brief status of the major breeding programmes on the following 4 questions:

1. What hybrids have been produced and for what purpose
2. Where are they used
3. How are they used
4. What are the prospects

The main breeding programmes considered in this chapter are CARBAP (Cameroon), CIRAD (Guadeloupe), EMBRAPA (Brazil), FHIA (Honduras) and IITA – NARO (Uganda). Further information on several improved hybrids can be found on the ProMusa information platform at: http://www.promusa.org/Diversity+of+banana+cultivars+portal

12.1.4.1 Breeding at CARBAP (Cameroon)

(the following section was provided by P. Noupadja, CARBAP)

The table below describes the hybrids produced at CARBAP to date, including information on the purpose they were produced, where they are used, how they are used and prospect for further breeding.

Table 12.1. *List of hybrids from CARBAP and descriptions of use to date.*

Hybrid name	Why they were produced	Where is this hybrid used	How is this hybrid mainly used	Prospects of further breeding	Comments or notes on the hybrid
CARBAP 568	Resistance to black leaf streak; dwarfness	Cameroon, Congo, Democratic Republic of Congo, Benin, Togo, Ghana, Central African Republic	Cooking	Improve pulp quality; Breed for resistance to weevil	Dwarf type, early maturity, poor quality of pulp
CARBAP 838	Resistance to black leaf streak	Cameroon, Congo, Democratic Republic of Congo, Benin, Togo, Ghana, Central African Republic	Cooking	Improve pulp quality; Breed for resistance to weevil	Hybrid from French sombre, small fingers, Firm pulp
CARBAP 969	Resistance to black leaf streak	Cameroon, Congo, Democratic Republic of Congo, Benin, Togo, Ghana, Central African Republic	Cooking	Improve pulp quality; Breed for resistance to weevil	Hybrid from French sombre
CARBAP K74	Resistance to black leaf streak	Cameroon	Cooking when green	Improve pulp quality; Breed for resistance to weevil	Secondary triploid hybrid (AAA) from Yangambi km5 and French sombre, Poor pulp quality when ripe; Good for flour when green.

12.1.4.2 Breeding at CIRAD (Guadeloupe, France)

(the following section was provided by J.P. Horry, CIRAD)

The original "reconstructive breeding" strategy developed by CIRAD has been applied to develop triploid hybrids of dessert bananas, both mono (AAA) and interspecific (AAB and ABB). Reconstructive breeding aims to identify good specific combining abilities between diploids and doubled-diploids as donors of diplo-gametes. In addition, it aims to maximize heterosis in the triploid progenies. Parental lines are selected according to their agronomic and fruits characteristics (linked to the type of banana –cooking or dessert, to be developed), their behaviour with respect to diseases and pests, and their male and female fertility at diploid and tetraploid level. Furthermore, the in-depth knowledge of the available genetic resources and the understanding of the relationships between ancestral and cultivated varieties allow selecting the pools of parent lines according to the desired results.

Breeding for AAA dessert banana

Breeding for dessert bananas is conducted in Guadeloupe at CIRAD's research station in partnership with the French West Indies growers association and its technical institute. Around one thousand hybrids are produced and evaluated annually. Objectives are to develop pest and disease-resistant or -tolerant banana hybrids for the export market to Europe and for domestic markets. Beside resistance to Sigatoka leaf diseases, Fusarium wilt and nematodes, selected hybrids must combine agronomic performance and fruit qualities. Moreover, a strong emphasis is put on fruit quality and postharvest behaviour regarding the heavy constraints required for the export market.

At CIRAD, where the "reconstructive breeding" approach has been prioritized for several years, progress has been made in developing AAA dessert bananas. Several hybrids recently obtained have been released for large-scale evaluation to banana growers in the Caribbean (Dominique, St Vincent, St Lucia, Cuba) and in Australia to supply domestic markets, and two of them (CIRAD 916 and CIRAD 918) are under evaluation in IMTP trials. Four of these hybrids have been evaluated in French Guyana and two of them, CIRAD 916 and 918, have already been adopted by farmers.

CIRAD 925 is to date the most promising hybrid created, combining nearly all the qualities to respond to the export industry requirements. The elite hybrid is presently under large scale evaluation in grower's fields in Guadeloupe and Martinique to validate its adaptation within the different steps of the export industry sector.

The understanding of the relationships between ancestral and cultivated varieties, allows now more relevant choices of parental combinations. In recent years, focus at CIRAD has been on the use of Mlali type AAcv accessions, putative ancestors of Cavendish and Gros Michel groups. Selected Mlali diploids were tested after chromosome doubling in crosses with AA accessions of the Khai cluster (the other ancestor of these groups). Among the progenies, one individual was obtained, and despite its predictable susceptibility to Yellow Sigatoka, it looks incredibly like Lacatan, the giant and original form of Cavendish, both in terms of plant stature than in fruit quality, thus providing the proof-of-concept for this novel breeding approach.

The drawback of this approach is the lack of resistances within the Mlali group, and their scarcity in the Khai cluster, combined with low gamete fertility of AAcvrs. Therefore, CIRAD engaged in a pre-breeding program to develop fertile improved progenitors containing various sources of diseases resistant genes derived from crosses between edible diploid clones and wild relatives, linked with in-depth studies of the genetic determinism of major traits, combining segregating population analysis, GWAS and QTLs development.

Breeding for AAB/ABB dessert banana

Breeding programs for interspecific hybrids (AAB Silk and Pome, ABB Pisang Awak) aim at the development of new cultivars with Fusarium wilt- and Sigatoka diseases-resistance that retain the organoleptic qualities and productivity of the traditional landraces. The "reconstructive approach" was applied to interspecific breeding at CIRAD, and triploid hybrids were derived from the Kunnan landrace (ABcv), its genomic constitution suggesting that it would come from a cross between a *malaccensis*-derived edible diploid and

a *balbisiana* wild relative. Moreover, 'Kunnan' has been proved free from infectious eBSV, allowing its use in crosses. Natural AB clones are sterile but their AABB neo-allotetraploid counterparts obtained through colchicine treatment are both male and female fertile. Tetraploid 'Kunnan' was crossed with AA and BB accessions to generate a triploid. All crosses taken together, 70 hybrids have been field-tested, 61 of which involved wild diploids, *Musa acuminata* or *Musa balbisiana*. It is noteworthy that most of these hybrids bear parthenocarpic (edible) fruit, despite their wild parentage. A common feature within the progenies evaluated is the positive heterosis effect observed. For most characters, mean values observed are significantly superior to the average values of the parents, and in some cases exceed the value of the best parent.

In crosses with wild and edible disease resistant *malaccensis* derivatives, several AAB hybrids were obtained ranging from Silk-like to Mysore-like cultivars and with resistance to Fusarium wilt and Sigatoka leaf streak diseases. On the other side, in crosses with *balbisiana* accessions, several ABB hybrids developed were very similar to 'Pisang Awak' natural clones. The best selected hybrids are presently under evaluation in Guadeloupe.

Within a limited number of hybrids, it has been possible to select some outstanding hybrids from each cross: the AAB '2006-22/III9' with a Mysore morphology, the sweet-acid AAB banana hybrid '2005/25-L9', the Pisang Awak-like '2008/12-I6' and some other weighted bunches ABB hybrids. These promising hybrids need further physiological and physicochemical but might be in in the short term candidates for IMTP trials.

Breeding for cooking bananas

Breeding for cooking bananas follows the "pragmatic" approach and aims at the development of secondary triploid hybrids. CIRAD breeding for cooking-types is conducted in partnership with CARBAP, the ownership of the selected hybrids being shared between the two institutes.

The presence of endogenous integrated sequences of the BSV in the plantain genome and the release of infectious viral particles in the progenies following crosses has long been an obstacle to plantain breeding. However, these viral sequences behave as pseudo-genes and can segregate as heterozygous loci. Recombination between A and B chromosomes during meiosis allowed the development at CARBAP of triploid hybrids that are free from infectious eBSV, obtained from AAAB (primary tetraploids) x AA (primary diploids) crosses. Marker-assisted selection developed by CIRAD (PCR, southern blot) is now exploited to release new cooking hybrids free from any infectious eBSV. 'CARBAP K74' is the first elite plantain-like hybrid obtained at CARBAP in 2012.

12.1.4.3 Breeding at EMBRAPA (Brazil)

(the following section was provided by E. Perito Amorim, EMBRAPA)

Brazilian banana production differs from other global contexts since its main cultivars belong to the AAB Pome/Prata subgroup, in particular, Prata-Anã. Also, especially in the north and northeastern regions of the country, Pacovan and plantain cultivars are also present in Brazilian plantations. These two cultivars, together, make up approximately 80% of the banana cultivated area in Brazil (500,000 ha). The Cavendish subgroup, represented in Brazil by the cultivars Grande Naine, Nanica and Nanicão, are mostly planted in the Southeast region (Vale do Ribeira, São Paulo State) and in the South of Brazil (Northeast of Santa Catarina and Paraná States). The Prata Anã and Pacovan cultivars were developed by EMBRAPA Cassava and Fruits through identification in banana growing areas, evaluation of agronomic potential, national competition assays and other recommendations. As with many other crop species, bananas and plantains are attacked by many phyto-pathogens such as fungi, nematodes and insects. The most important fungi are those causing yellow Sigatoka disease (*Mycosphaerella musicola* Leach), black leaf streak disease (*Mycosphaerella fijiensis* Morelet) and Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*). Nematodes which lead to huge losses are *Radopholus similis* and *Meloidogyne incognita*, and the most important insect in production areas is the banana weevil borer (*Cosmopolites sordidus*). In Brazil, specifically, Fusarium wilt is the major constraint facing Brazilian banana production, hindering expansion to other areas.

The main cultivars used by banana growers (Bananas: Prata-Anã, Pacovan, Silk, Grande Naine and plantains: Terra Maranhão, Terrinha and D'Angola) are susceptible to yellow Sigatoka and black leaf streak diseases. With regard to Fusarium wilt, Grande Naine and plantains are resistant, Silk is highly susceptible and the remaining cultivars are moderately susceptible. In the specific case of plantains, borer weevil and nematodes are the main limiting factors as far as maintaining the plants in the field is concerned, since they are highly susceptible.

EMBRAPA carries out the only banana breeding program in Brazil, initiated in 1976, by developing its germplasm collection. It is a product of national and international collections. This program has developed the following cultivars through crosses: BRS Caprichosa, BRS Garantida, BRS Japira, BRS Pacovan Ken, BRS Preciosa, BRS Princesa, BRS Tropical, BRS Vitória, BRS Pioneira, BRS Platina and BRS Pacoua. EMBRAPA has also recommended three cultivars from collections or identification of mutations: BRS Conquista, BRS Pelipita, BRS Thap Maeo and BRS SCS Belluna.

These cultivars are used by growers in different Brazilian regions, mostly in the North and Northeast regions, where these same cultivars are responsible for the income of small producers, especially in areas where the use of technology is still at its infancy. It is worth highlighting that the two most important Brazilian cultivars – Prata Anã and Pacovan – which together are responsible for approximately 80% of the cultivated area were identified and recommended by the banana genetic breeding program at EMBRAPA.

EMBRAPA uses three main strategies of conventional breeding:

1. Crosses between wild diploids to develop improved diploids resistant to main pests and diseases and good agronomic characteristics: EMBRAPA has a collection with 30 improved diploids, all resistant to Fusarium wilt and yellow Sigatoka, and most of them, also resistant to black leaf streak
2. Crosses between improved diploids and commercial triploid cultivars (Prata type, Silk and Cavendish) to develop tetraploid hybrids
3. Crosses between improved diploids and tetraploid hybrids to develop triploid cultivars: This strategy is the main current focus and hundreds of genotypes are being evaluated in the many experimental areas of EMBRAPA.

As far as biotechnological tools are concerned, EMBRAPA has many different strategies which support the conventional breeding program:

1. Plant x biotic factors interaction studies (*Mycosphaerella musicola* / *Mycosphaerella fijiensis* / *Fusarium oxysporum* f. sp. *cubense*): Potential candidate genes associated to resistance to these three diseases have been identified via NGS (Next Generation Sequencing) - RNA-seq techniques. These genes are being validated through RT-qPCR for further use in cisgenics and marker-assisted selection (MAS). In the case of cisgenics, EMBRAPA has identified tissue-specific promoters (leaf and root). New interactions, however, are also being planned (nematodes and borer weevil)
2. *Musa* x abiotic factors interaction studies, especially concerning drought tolerance and post-harvest are ongoing using a proteomics approach. Many candidate genes have been identified as being associated in metabolic networks as hub proteins involved in drought tolerance and will be sought out for use in MAS. Similar work is being carried out for fruit ripening and finger drop, especially for Prata type cultivars
3. Diversity and genetic structure of *Mycosphaerella musicola*, *Mycosphaerella fijiensis*, *Fusarium oxysporum* f. sp. *Cubense*, *Meloidogyne incognita* (root-knot nematode) and *M. javanica* populations with the objective of creating a detailed map of the distribution of these pests and diseases in main banana producing areas in Brazil. This work will be followed by the identification of most aggressive or virulent populations for use in inoculations aiming for the early selection of resistant or tolerant genotypes. Protocols for early selection were developed by EMBRAPA and are being applied

4. Duplication of chromosomes from wild and improved diploids: Many diploids were duplicated and the number of chromosomes confirmed by flow cytometry and cytogenetics. These auto-tetraploids were characterized for a series of agronomic characteristics and identified as promising, which were then crossed with diploids for the development of secondary triploids and are now under evaluation in the experimental areas of EMBRAPA
5. Somatic embryogenesis and cell suspensions: EMBRAPA has developed protocols for generating cell-suspensions and plant regeneration. Work with molecular markers show that these suspensions give rise to genetically stable plants
6. Induction of mutation using gamma rays and anti-mitotic agents: The focus here is to identify short stature plants and recently induce mutants with resistance to Fusarium wilt and to yellow Sigatoka and black leaf streak. Results so far seem promising.

Preventive breeding is also an objective of EMBRAPA, especially regarding *Fusarium oxysporum* f. sp. *cubense* Tropical race 4. EMBRAPA will send its elite germplasm for testing at PRI / Plant Research International - University of Wageningen – The Netherlands - and the Department of Agriculture, Fishery and Forest in Australia (DAFF), which are our main partners in this task.

EMBRAPA also has a partnership with the BGPI research unit (Biology and Genetics of Plant-Pathogen Interactions) of CIRAD- Montpellier, France, in activities to identify BSV species in the banana germplasm collection at EMBRAPA and also main banana producing areas in Brazil, since this data is practically unknown and necessary for main germplasm exchange activities.

Since EMBRAPA´s breeding program is one of the oldest, with great history and knowledge acquired/ generated, EMBRAPA is in the forefront of banana breeding and has great potential to collaborate with international partners focusing on developing cultivars resistant to main pests and diseases possessing sensorial characteristics aligned with producers and consumers demands.

It is worth mentioning that since Brazil is a continental country, it has a wide range of edaphoclimatic conditions which allows for the evaluation and selection of genotypes in the many different environments. EMBRAPA has a broad network of partners and its own bases able to test new hybrids from regions with elevated pluviometric indices to dry areas, environments with temperate climate to tropical climate, areas from sea level to high altitudes, areas with bananas as monoculture or in consortium, among many other contrasts.

Table 12.2. *List of hybrids from EMBRAPA and descriptions of use to date.*

Hybrid name	Why they were produced	Where is this hybrid used	How is this hybrid mainly used	Prospects of further breeding	Comments or notes on the hybrid
BRS Tropical	Resistant to yellow Sigatoka and tolerant to Fusarium wilt	Brazil	Dessert	Convert to triploid and Improve resistance to black leaf streak and Fusarium wilt (BRS Tropical crosses with improved diploid). Select Dwarf type	Silk type (AAAB)
BRS Princesa	Resistant to yellow Sigatoka, black leaf streak and Fusarium wilt	Brazil	Dessert	Convert to triploid (BRS Princesa crosses with improved diploid). Select Dwarf type	Silk type (AAAB)

Hybrid name	Why they were produced	Where is this hybrid used	How is this hybrid mainly used	Prospects of further breeding	Comments or notes on the hybrid
BRS Platina	Resistant to yellow and Fusarium wilt and moderately resistant to black leaf streak	Brazil	Dessert	Convert to triploid (BRS Platina crosses with improved diploid). Improving shelf life	Pome/Prata type (AAAB)
BRS Pacoua	Resistant to yellow Sigatoka and Fusarium wilt and moderately resistant to black leaf streak	Brazil	Dessert	Convert to triploid (BRS Pacoua crosses with improved diploid). Select Dwarf type	Pome/Prata type (AAAB)
BRS Caprichosa	Resistant to yellow Sigatoka, black leaf streak and Fusarium wilt. Moderately resistant to Weevil and nematodes	Brazil	Dessert	Select Dwarf type	Pome/Prata type (AAAB)
BRS Garantida	Resistant to yellow Sigatoka, black leaf streak and Fusarium wilt. Moderately resistant to Weevil and nematodes	Brazil	Dessert	Select Dwarf type	Pome/Prata type – Pome (AAAB)
BRS Vitória	Resistant to yellow Sigatoka, black leaf streak and Fusarium wilt	Brazil	Dessert	Convert to triploid (BRS Vitória crosses with improved diploid). Select Dwarf type	Pome/Prata (AAAB)
BRS Japira	Resistant to yellow Sigatoka, black leaf streak and Fusarium wilt. Moderately resistant to Weevil	Brazil	Dessert	Convert to triploid (BRS Japira crosses with improved diploid). Select Dwarf type	Pome/Prata type (AAAB)
BRS Pacovan Ken	Resistant to yellow Sigatoka, black leaf streak and Fusarium wilt. Moderately resistant to Weevil	Brazil	Dessert	Select Dwarf type	Pome/Prata type (AAAB)
BRS Preciosa	Resistant to yellow Sigatoka, black leaf streak and Fusarium wilt	Brazil	Dessert	Select Dwarf type	Pome/Prata type (AAAB)
BRS Pioneira	Resistant to yellow Sigatoka and Moderately resistant to Weevil	Brazil	Dessert	-	Pome/Prata t ype (AAAB)

12.1.4.4 Breeding at FHIA

The limited information in Table 12.3 below is extracted from a presentation made by FHIA at FAO, Rome, on 23 September 2014. Links to Promusa pages on the FHIA hybrids are also provided.

Table 12.3. *List of hybrids from FHIA and descriptions of use to date.*

Hybrid name	Why they were produced	Comments or notes on the hybrid
FHIA 01	Resistant to Sigatoka and FOC-TR4	http://www.promusa.org/FHIA-01
FHIA 03		http://www.promusa.org/FHIA-03
FHIA 17		http://www.promusa.org/FHIA-17
FHIA 18		http://www.promusa.org/FHIA-18
FHIA 20		http://www.promusa.org/FHIA-20
FHIA 21		http://www.promusa.org/FHIA-21
FHIA 23		http://www.promusa.org/FHIA-23
FHIA 25	Resistant to Sigatoka and *Foc*-TR4	Pome type http://www.promusa.org/FHIA-25
SH4037	PreBreeding	

12.1.4.5 Breeding at IITA (Nigeria/Uganda)

(the following section was provided by R. Swennen, IITA)

Table 12.4 below describes the hybrids produced at IITA to date, including information on the purpose they were produced, where they are used, how they are used and prospect for further breeding. Countries that received the material in 2014-2015 are listed. It is still too early to speak of use, as material has only been delivered for testing. Only in IITA are some of the hybrids that are being used by farmers.

Table 12.4. *List of hybrids from IITA and descriptions of use to date.*

NOTE: all hybrids below are IITA hybrids, but NARITAs are NARO/IITA hybrids.

Hybrid name	Why they were produced	Where is this hybrid used	How is this hybrid mainly used	Prospects of further breeding	Comments or notes on the hybrid
TMP2x 2829-62; TMP2x 1297-3	Host plant resistance to black leaf streak	Nigeria, Cameroon, Uganda	Plantain derived; breeding	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
TMp2x 9128-3	Very long pendent bunch	Nigeria, Uganda, Puerto Rico, Tanzania	breeding	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
BITA 2; BITA 3; PITA 14	Host plant resistance to black leaf streak, yield	Nigeria, Cameroon, Ghana, Puerto Rico	Plantain-like derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration

Hybrid name	Why they were produced	Where is this hybrid used	How is this hybrid mainly used	Prospects of further breeding	Comments or notes on the hybrid
PITA 1; PITA 4; PITA 5; PITA 7; PITA 8	Host plant resistance to black leaf streak, yield	Nigeria, Ghana	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
PITA 2; PITA 16	Host plant resistance to black leaf streak, yield	Nigeria, Puerto Rico	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
PITA 3	Host plant resistance to black leaf streak, yield	Nigeria, Ivory Coast	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
PITA 6; PITA 11; PITA 13; PITA 18	Host plant resistance to black leaf streak, yield	Nigeria	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
PITA 9	Host plant resistance to black leaf streak, yield	Ghana	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
PITA 10	Host plant resistance to black leaf streak, yield	Puerto Rico	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
PITA 12	Host plant resistance to black leaf streak, yield	Nigeria, Ghana	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
PITA 17; PITA 21; PITA 22; PITA 23; PITA 24; PITA 26; PITA 27	Host plant resistance to black leaf streak, yield	Nigeria, Ivory Coast, Ghana, Cameroon, Puerto Rico, Burundi, Rwanda, Congo, Benin, Comoros	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration

Hybrid name	Why they were produced	Where is this hybrid used	How is this hybrid mainly used	Prospects of further breeding	Comments or notes on the hybrid
PITA 25	Host plant resistance to black leaf streak, yield	Nigeria, Comoros, Congo	Plantain derived; breeding; Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance and adding dwarfism and higher provitamin A	Fields established to gather data for registration
NARITA 1; 2; 4; 5; 6; 7; 11; 12; 13; 14; 15; 17; 18; 19; 20; 21; 22; 23; 24; 25; 26	Host plant resistance to black leaf streak, yield	Uganda, Tanzania	Cooking	Integrating/ confirmation for weevil/nematode/ Fusarium resistance	Multilocational trials starting in Uganda and Tanzania in 5 locations, evaluation by consumers planned in these 5 sites
NARITA 3; 8; 9; 10; 16	Host plant resistance to black leaf streak, yield	Uganda, Tanzania	Juice/beer	Integrating/ confirmation for weevil/nematode/ Fusarium resistance	Multilocational trials starting in Uganda and Tanzania in 5 locations, evaluation by consumers planned in these 5 sites

12.1.5 Biotechnologies and breeding

In a comprehensive overview, Ortiz and Swennen reviewed the recent advances in biotechnology and their actual and future applications in banana breeding (Ortiz and Swennen 2013). This section is limited to the biotechnological tools that are routinely used in most banana breeding programs.

12.1.5.1 Tissue culture

In vitro micropropagation of banana is now widely used for germplasm exchange and also to supply clean planting materials to farmers and intensive cropping systems. Even if tissue propagules are more expensive than traditional suckers, the yield increase may compensate for the investment. Applied in most breeding programs, *in vitro* embryo rescue assists in enhancing germination of banana seeds resulting from controlled pollination involving cultivars, often containing abnormal embryos or absent endosperm.

12.1.5.2 Cytogenetics

Cytogenetics research in *Musa* has been developed since the early 1920s to elucidate the genetic structure of the polyploid complex, from chromosome numbering to the study of chromosome pairing during meiosis. The development of *in situ* hybridisation (FISH and GISH) allows for a precise deciphering of the chromosome rearrangements and pairing between the homologous A and B genomes. In a recent study, Jeridi showed that gamete sterility in AB clones is probably due to incomplete chromosome pairing at meiosis: the authors distinguished two subgroups of pairing chromosome behaviour, one of eight chromosome sets displaying high (but not complete) pairing affinity, suggesting a high degree of homology between A and B chromosomes, and a second of three chromosome sets presenting low pairing affinity, indicating a lower degree of homology (Jeridi et al. 2012). This result also supports the hypothesis that recombination does occur between A and B genomes for at least eight chromosomes of eleven. Flow cytometry is routinely used to characterize genetic resources in genebanks of parents and progenies in most breeding programs.

12.1.5.3 DNA markers

In recent decades, several genomic tools have been successfully used to decipher the complexity of the *Musa* genepool. Molecular markers have not only proven to be effective to distinguish between genotypes, but moreover have provided a clear understanding of the history of the domestication of the crop, from the ancestor wild species to the present day cultivars. The acquired knowledge of the probable diploid ancestors of most triploid cultivars allows us to build breeding schemes that mimic the sequence of crossings and selections that occurred over several millennia. DNA markers (SSR, DArTs) are now routinely used to characterize genebank materials, and are used in breeding program to check the conformity of breeding materials.

12.1.5.4 Genomics

Recent advances in *Musa* genomics, from the first complete sequence of the *Musa* genome published in 2012 to the ongoing re-sequencing of hundreds of genotypes will increase the efficiency of banana breeding. The *Musa* genome can now be deeply explored for the characterization of desirable genes involved in important agricultural traits as a prelude to their use in marker assisted selection. Priorities for the GTG of MusaNet in this area include improved gene annotation and elucidation of gene function through analysis of gene expression in specific conditions, mutation/tilling, GM approaches, proteomics and metabolomics. The very fine structure of the genome, the arrangement of the chromosomes and of the DNA sequences can now be studied with an unprecedented precision, adding to the characterization of the genetic complex and the understanding of the evolutionary pathways. For banana improvement, this approach is particularly important in a biological context of gamete sterility (and difficulties to get large quantities of seeds) for which use of recombination and implementation of introgression strategies remain the greatest challenge.

12.1.5.5 Marker assisted selection (MAS)

Marker assisted selection is only just beginning in banana breeding. From the recent and future advances in genomics, MAS will eventually enhance and accelerate genetic improvement in banana. However, presently, its main application relates to the early selection of non-infectious eBSV alleles in interspecific crosses involving *balbisiana*-derived material.

12.1.5.6 Genetic engineering

Genetic engineering of bananas began 20 years ago, with expectations to accelerate banana breeding by directly improving sterile cultivars that appear untreatable through cross-breeding. The main objectives are to increase the micronutrient content to alleviate malnutrition in East African countries and to incorporate traits that are lacking in the *Musa* genepool. Among the latter are the genes for resistance to viruses and *Xanthomonas* wilt, for which no reliable resistance source seems to exist. Moreover, strategies for the acquisition of resistance to other diseases by genetic transformation are now available, like the use of protease inhibitors and *Bacillus thuringiensis* genes to develop resistance to weevils and nematodes, or the identification of resistant genes such as chitinases, antifungal proteins, etc., against Sigatoka diseases and Fusarium wilts.

The goal is to incorporate in the genome relevant DNA-sequences that should express a desired improvement. While the incorporation of genes has been proven to be quite feasible, its desired expression depends on the extraordinary complex dynamics in the transciptome and proteome. Progress has recently been made in that domain for model plants such as *Arabidopsis*, and it can be expected that genetic engineering of bananas will in the coming years integrate the necessary precise mechanisms for the desired expression of genetically modified cultivars.

Recent developments of genome editing with engineered nucleases, such as CRISPR technology, which does not involve taking genes from one organism and implanting them in another, appears to be a promising approach for banana breeding in the near future. In this respect, an interesting outlook will be that such genetically modified diploids could enter the breeding schemes at appropriate phases.

12.1.5.7 Induced mutation techniques

Work has also been carried out using mutation to expand the range of *Musa* diversity available for breeding. Spontaneous somatic mutations have long played an essential role in the speciation and domestication of bananas. In an effort to mimic this natural occurring process, mutagenic agents such as radiation and certain chemicals have been used to induce mutations at a higher frequency and generate genetic variation from which desired mutants may be selected. Several variants and putative mutants have been identified at the International Atomic Energy Agency (IAEA) in Austria for release or further confirmation trials. One example is a the variety Klue Hom Thong KU1 from Thailand, which was obtained by treating tissue cultures with gamma rays at 2.5 krad (25 Gy), and was selected for its larger bunch size and cylindrical shape. Even though the traditional shoot-tip mutation-induction techniques applied to genetic improvement has produced some useful mutants, there are some limitations, e.g. treated shoot tips show a high degree of chimerism and because of the random nature of mutation induction, many plants need to be screened, which can be expensive, laborious and site-specific. But now we know that to overcome chimerism, embryogenic cell suspension (ECS) is the material of choice (as embryos are formed from single cells). Early mass screening techniques (*in vitro* or in greenhouse) are being used to increase efficiency before validating results under field conditions (Roux, 2004). Thanks to recently developed next generation sequencing techniques, there is an increased interest in collecting mutants to understand *Musa* gene structure and function.

SECTION 12.2 GENETIC IMPROVEMENT – WHERE WE WANT TO GO

The products of existing improvement programmes, drawing on sources of biotic and abiotic stress resistance from wild and edible genotypes, are still not meeting important criteria, such as (1) adaptation to recurring extreme weather-conditions, such as drought and cold, mainly in peripheral areas of the banana biotope, and (2) widely and/or regionally acceptable fruit quality.

12.2.1 Addressing abiotic stresses

Variation among wild and edible *Musa* species offers a wide spectrum of promising phenotypes. For instance, the ecology of various wild species suggests that sources of resistance to abiotic stresses exist in Eumusa along the northern altitudinal periphery of its distribution, including mechanisms for tolerance to cold (*M. sikkimensis, M. basjoo, M. thomsonii*), water-logging (*M. itinerans*), and drought (*M. balbisiana*, *M. nagensium*). The physiological and genetic background of the quite diverse ex-section Rhodochlamys members, adapted to monsoon conditions, should also be investigated. Recent collecting expeditions in northern India, Malaysia and Indonesia suggest that other poorly known or unexplored areas of diversity (such as cultivars in the species *M. balbisiana* or in the *acuminata* subspecies) are likely to harbour other interesting agronomic characteristics. Unfortunately, many such underexplored specimens (with the remarkable exception of *M. balbisiana*) are hardly expressing their phenotypic potential outside their usual habitat, as is the case in the common lowland banana collections, or they even disappear after a few years. And inter-specific F1 progeny derived from crossing such specimens with common wild AA/BB parents would not be fertile in most cases. *In situ* extraction of DNA in view of further analyses and eventual integration in genetic improvement may be a more promising prospect.

Breeders are well aware of this potential, but there is the need for a programme and funding of such special operations.

A remarkable exception is the adaptation of some triploid groups such as Cavendish, grown under a large range of latitudes and ecologies, an adaptability that is retrieved in the edible *acuminata* cultivars of East Africa and neighbouring islands. Genetic diversity in these diploids is very low, suggesting a very narrow genetic base; however, they are cultivated from sea level to the slopes of Mount Kenya, at more than 1000 m above sea level. This suggests that genetic resources adapted to extremely different ecologies are available for the breeders, even within the *acuminata* genetic stocks.

12.2.2 Addressing the desired plant phenotype and fruit quality

Although there are now many hybrids that have been developed to address issues such as pests and diseases, fruit quality has often been neglected. Consequently, the products are generally not meeting the fruit quality to which the diverse local consumers are accustomed. Each region and each country will have specific needs and requirements.

The term “fruit quality” should be considered in its broadest sense, thereby not only meaning “organoleptic quality” but also the entire chain from farm to market. For example, the truck transport of bunches over relatively long stretches of local roads means that only physically resistant fruits are convenient on the markets. Early ripening and premature fruit dehiscence are also rejected. Fruit post-harvest qualities are even more constraining for exportation, where fruits must resist several weeks of shipment.

Consequently, the construction of synthetic diploids in breeding programs should not only catch the sources of disease/pest resistance, but also include fruit quality in its broadest sense. Such range of qualities apparently exists among the numerous edible AA but has been poorly explored from a genetic perspective. Characterization of fruit quality in the diploid genetic pool and the elucidation of the genetic basis are the priorities to address and monitor these traits in breeding programs.

Yet only a fraction of the genetic diversity in the genus *Musa* is being used, even within *balbisiana* and *acuminata* species. Breeding programmes need to broaden their genetic base to address the numerous challenges of banana breeding, considering both pests and diseases, fruit quality (organoleptic and post-harvest) and agronomic features (e.g. yield, adaptation to miscellaneous and changing environments).

SECTION 12.3 GENETIC IMPROVEMENT – HOW WE WILL GET THERE

12.3.1 Preamble

A fully concerted banana genetic improvement programme on global scale cannot be realized. Such a programme would imply that the participants constantly share their detailed operational strategy for each end-product at which they are aiming: the satisfactory hybrid. The programmes would probably not have problems in the ideal situation of an assured abundant financing, but that is far from the case for the banana crop. Banana improvement programmes therefore operate in a competitive configuration which obliges them to protect their precise methodology in the hope that ‘their’ product, when successful, would produce a return to finance the continuation of their efforts. One can use the term “property rights” in this case.

Nevertheless, two phases of the improvement open the prospect for sharing at global scale:

- The pre-breeding phase, with a common sharing of the basic plant material (and its performance) such as selected edible diploids and even partially improved diploids
- The post-breeding phase, with the testing of hybrids in different environmental and cultural conditions. The IMTP principle could be used as a convenient instrument.

Both phases could be facilitated via coordination at global scale.

12.3.2 The Douala Workshop on Musa Breeding, 28-30 October 2013

For the first time in the history of banana research, a representative group of scientists involved in genetic improvement came to an agreement on collaborative activities during the workshop “Multi-centre Planning on Banana / Plantain Improvement” in October 2013 at Doula, Cameroon.

The workshop was co-organized by CARBAP, Bioversity and IITA and financed by the Bill and Melinda Gates Foundation (BGMF) and the CRP-RTB. The major actors were the leading *Musa* research and breeding programmes and experts from allied crops and disciplines.

Quoting from the workshop report, “the primary objective of the workshop was to establish a consensus opinion on the long-term strategy (and key priorities) for “accelerated” banana crossbreeding focused on outputs for Africa that would also have relevance for other regions. In addition, the workshop aimed to define the respective roles of various interested parties, and establish the framework for a concept note (focusing on breeding plantain-like hybrid cultivars)”.

The Concept Note, entitled “Program for improved plantain for Sub-Saharan Africa (PIP-SSA), a global breeding partnership”, covers all the aspects of such an undertaking at a global scale. The pre-and post-breeding tasks, which call for advanced sharing of material and results were particularly developed. Collaboration for the breeding activities was outlined as far as possible and the means for sharing the rapidly growing knowledge on molecular genetics (omics datasets, genetic information on genes-traits correlation, etc.) for their integration in breeding programmes were systematically proposed.

While it is mainly focused on the important subgroup of AAB Plantain in Africa, the Concept Note can indeed be used as a model for similar collaborative programs in all other regions.

The table below summarizes the future objectives within the breeding programmes.

Table 12.5. *Objectives and proposed actions regarding genetic improvement.*

Objectives	Proposed actions
1. Meeting breeders’ needs of parental stocks to address biotic and abiotic stresses	• Explore and collect genetic stocks in poorly explored areas and diverse ecologies • Characterize and evaluate genetic stocks of potential interest in diverse ecologies • Make available a wide spectrum of genetic resources to breeders, including outsider specimens of interest
2. Improving knowledge of fruit quality in parental stocks	• Characterize fruit qualities of diploid germplasm • Foster research on the genetics of fruit quality traits
3. Improving pre-breeding at a global scale	• Share knowledge among the breeders of the performance of genetic stocks used in breeding • Facilitate the exchange of basic genetic stocks of breeding interest • Encourage and facilitate the sharing of improved diploids between breeding programs
4. Improving the evaluation and adoption of improved varieties	• Establish a secure network for the evaluation of novel varieties, such as IMTP, including the respect of IP rights of the breeders • Evaluate hybrids under diverse environmental and cultural conditions

SUMMARY OF ACTIONS

SUMMARY OF ACTIONS

This section is a summary of the proposed actions listed in the tables at the end of each chapter (in *How will we get there*), organized into the five thematic groups (TG) of MusaNet (Diversity, Conservation, Evaluation, Information and Genomics). One of the main objectives of MusaNet is to implement the Global Strategy through its projects and activities. Therefore these tables will allow MusaNet members to focus their efforts on the activities planned within their TG over the next 5 years, as well as to see how those activities link to the other TGs in order to foster more collaboration.

For a description of Musanet, see the Introduction and Annex A. More information can be found on www.musanet.org.

Diversity TG

Objectives	Proposed actions
Fully assess the diversity of *M. acuminata* and *M. balbisiana*	• Set up collecting missions to: Myanmar, Extreme North India, Indonesia New Guinea, East Africa, Near Oceania • Study the diversity of wild genepools with molecular markers
Refine the taxonomy of triploid cultivars	• Identify subgroup discriminative descriptors through the multi-environment characterization of the TRC • Identify subgroup-specific descriptors through the extensive characterization of targeted subgroups
Revise the taxonomy of diploid cultivars	• Characterize the accessions composing the molecular clusters and assess if they compose subgroups • If so, agree on subgroups names
Explore AB diversity	• Perform a survey of the AB in *ex-situ* collections (with descriptors and photos) • Molecular analyses of these AB
Assess which of the descriptors are robust across environments	• Multi-location characterization of the TRC • Statistical analysis of the results obtained
Identify subgroup-specific descriptors	• Organize regional workshops dedicated to specific subgroup e.g. East Africa for EAHB
Facilitate the identification of cultivars – wild types	• Update Musa.ID
Optimize use of past work with SSR	• Pursue the molecular characterization of *Musa* diversity with SSRs to enrich existing databases and reach a molecular picture of the whole *Musa* diversity
Molecularly differentiate cultivars within subgroups	• Test new techniques available • Investigate other approaches (e.g. epigenetic)
Develop and publish catalogues on current diversity held in collections	• Publish catalogues of *Musa* diversity at CARBAP, USDA, the Philppines (UPBI) and as part of the TRC project.

Conservation TG

Objectives	Proposed actions
	***Ex situ* conservation**
Improve effective management of *ex situ* collections and enhancement of services	• Create a platform for effective information exchange and sharing of methods, techniques and experiences between collection managers • Update the 2008 Regeneration Guidelines • Develop field management guidelines including specific information on groups such as wild species management and ecological regions • Develop a new set of technical guidelines for the full range of activities in lab management including tissue culture establishment • Develop guidelines on collecting and acquiring new materials on missions in a collection • Develop academic training in plant genetic resources management and increase capacity building for virus indexing and molecular characterisation.
Identify and set up a global network of partners with specific responsibilities for conservation of the *Musa* genepool	• Strengthen the global network of partners with specific responsibilities for conservation of the *Musa* genepool including the safety duplication. • Improve characterization (phenotyping and genotyping) of germplasm in all collections to allow curators to make decisions on rationalization of accessions. • Introduce missing diversity to ensure full coverage at national, regional and global level. • Set up more locations of international field planting or *in vitro* culture conservation, safer for germplasm duplication and effective for *Musa* distribution. • Rationalisation of national collections based on improved characterization (phenotyping and genotyping) of germplasm.
Identify and set up a global core collection of *Musa* biodiversity in several designated sites for in perpetuity conservation	• Establishment of a global reference field collection (TRC) integrating subsets that represent specific parts of the diversity held by different collections. • Establish partnership agreements with regional and national field collections for complementary responsibility sharing to preserve global core accessions • Global core accessions duplicated *in vitro* and in cryopreservation at the ITC. • Targeted collecting and duplication of unique accessions from national collections, increasing the coverage of the known *Musa* diversity in the ITC collection
Increase access and targeted use of the ITC collection	• Link between ITC and MGIS database to create feedback mechanism for information on the ITC collection germplasm exchange and use • Promote the use of the on-line ordering tool running on MGIS for the global ITC collection. There is a new MGIS website with more user friendly functions for ordering accessions. • Field verification, morphotaxonomical characterization , flow cytometric ploidy determination and genotyping of the ITC collection to ensure the genetic integrity and improve the documentation status of conserved accessions • Identification of accession 'subsets' expressing certain desirable traits of interest for potential user groups • ITC to proactively distribute germplasm to collections with specific interests for specific regions, and indicate these as subsets in MGIS
Increase awareness of the need for high health status germplasm	• Update the Technical Guidelines for the Safe Movement of *Musa* Germplasm to incorporate newly discovered viruses and the latest indexing methods. Update disease Factsheets
Improve the efficiency of virus indexing protocols	• Review current virus indexing protocols to highlight deficiencies and inefficiencies
Seek a consensus on the risks of distribution of integrated, activable BSV in germplasm	• Bioversity to develop a position on the movement of germplasm with integrated, activable BSV based on the relative risks and advantages to the recipient country, and the responsibility of the germplasm supplier
Secure the long-term conservation of the entire ITC collection	• Cryopreserve the entire ITC collection • Safety-back up of the entire cryopreserved collection at off-site location (IRD, France)
Expand long term conservation capabilities by seed banking	• Explore the feasibility of seed conservation for preserving the wider wild diversity as complementary approach • Assess the diversity within/between populations, to make decisions on how many seeds to collect so that they can be conserved and distributed. • Develop a Global Musa Seed Bank to conserve and distribute seeds under the ITPGRFA

Objectives	Proposed actions	
	***In situ* and on farm conservation**	
	In situ	**On farm**
Map the distribution of CWRs and landraces in primary and secondary centers of diversity (potential sites - South East Asia (India) and the Pacific Islands)	• Establish and map the distribution of all taxa at all scales • Determine the threatened status of each taxon and red listing of highly endangered CWRs • Collecting and sharing traditional knowledge and uses linked to all wild *Musa* species	• Build regional databases of *Musa* landraces (with characterisation and evaluation data, indigenous knowledge, digital photo databases, and geo-referenced locations) • Develop distribution maps of landraces for analysis of geographic patterns • Identify geographic specific traits, i.e. traits which are specific to given areas (and their environmental constraints) • Establish and map the distribution of *Musa* landraces and farmers varieties • Determine the conservation status of all landraces and red listing those highly endangered
Establish institutional frameworks for the conservation of CWR and *Musa* landraces	• Develop national and International Agreements to allow for efficient and permanent safeguard of CWRs in protected areas • Strengthen linkages with National and International *Musa* Research conservation networks • Develop a territorial monitoring tool for CWR diversity conservation	• Develop national and International Agreements to allow for efficient and permanent safeguard of landraces in primary and secondary centres. • Facilitate national, regional and international networks to enhance local capacity for biodiversity information gathering and analysis. • Develop a territorial monitoring tool for landrace on farm diversity conservation
Promote farm conservation and utilization of landraces under changing climatic conditions		• Identify and promote the cultural value of local landraces • Facilitate national and international agreements on the conservation and use of landraces
Collect and establish DNA/RNA bank for all major landraces		• Carry out studies on genomics and associated trait characterization • Evaluate landraces against major stresses (drought, pest and diseases)

Evaluation TG

Specific objectives	Actions
Comprehensively assess currently available evaluation data	• Review of literature on evaluation of *Musa* genetic resources • Review of currently available phenotypic and genotypic evaluation data information in MGIS and other collection databases • Identify major gaps in knowledge in terms of traits and accessions
Standardize evaluation protocols	• Review currently available phenotyping/genotyping methodologies for evaluation of priority traits • Identify gaps in evaluation methodologies; identify for which traits and/or types of evaluation good protocols are not available • Develop and agree on a set of standard “best-practice” protocols for priority traits, and enter standardized traits/methods in Trait Ontology • Agree on a set of standard check genotypes for all trials • Identify a set of well characterized (climate, soil conditions, etc) reference trial sites

Specific objectives	Actions
Set up framework for data compilation and analysis	• Compile existing evaluation data in *The Global Agricultural Trial Repository of CCAFS* (AgTrials) (www.agtrials.org) • Ensure link between AgTrials and MGIS • Ensure link between AgTrials and Trait Ontology • Engage in global analyses for germplasm performance and GxE interactions
Share information and knowledge	• Make available and pro-actively share information and knowledge with the broader *Musa* research community and other users/stakeholders, in collaboration with MusaNet's Information Thematic Group and the global network ProMusa (www.promusa.org) • Make available a database search tool for information on different varieties that are being screened, such as agronomic, climatic and quality characteristics, in order to help priority setting in the regions

Information TG

Objectives	Proposed actions
Provide a set of tools to improve data quality and accuracy and facilitate data capture in collections with modern tools	• Release mobile application for data capture in the field (MusaTab) • Implement indicators of data completion for passport and characterization data • Provide regular training using the latest tools
Sustain development of a banana community portal for genepool banana diversity	• MGIS regularly updated with new collection and data • Link the accession level to cultivar level to retrieve information on the importance and value of the cultivar, including commercial and indigenous knowledge and potential post-harvest fruit quality, pests and diseases and other agronomic characteristic and performance
Complement documentation of accessions with phenotyping and evaluation data and facilitate data harmonization	• Record agronomical traits data from evaluation studies on germplasm material • Harmonize data with crop ontology for multi-crop passport data, anatomy, development stages, and agronomical traits

Genomics TG

Objectives	Proposed actions
Apply genomics tools to banana to better characterize genetic resources in collections and eventually support knowledge for breeding	• *Musa* Genomics annual workshop at Plant Animal Genome conference in San Diego. • Maintain and improve an integrated bioinformatics platform/Banana Genome Hub • Produce reference sequences for other species and subspecies • Characterize *Musa* germplasm genetic diversity through resequencing
Embrace the genomics of genebanks and aggregate omics data generated from germplasm material held in collections	• Banana Genome Hub regularly updated with new datasets • Interoperability fostered between MGIS, Banana Genome Hub and breeding resources such as Musabase

ANNEXES

ANNEX A.
NETWORKS AND PARTNERSHIPS

THE GLOBAL *MUSA* GENETIC RESOURCES NETWORK - MUSANET

The Global *Musa* Genetic Resources Network (MusaNet) was established in March 2011 as a global collaborative framework for effectively managing *Musa* genetic resources and a partnership of all key stakeholders, aiming at ensuring the long-term conservation of *Musa* genetic resources on a cooperative basis, and facilitating their increased utilization globally. It provides a collaborative framework to support the implementation of this Global Strategy for the Conservation and Use of *Musa* Genetic Resources.

The objectives of MusaNet are therefore in line with the strategy and are to:

- Ensure the secured conservation of the entire *Musa* genepool by assessing the diversity conserved and filling gaps, with an emphasis on threatened material
- Strengthen the capacity of partners for the cost-effective long-term conservation and management of germplasm collections and facilitate access to useful *Musa* genetic resources in improvement programmes and by other users
- Enhance the value of *Musa* genetic resources for breeding, through effective collaborative characterization, evaluation and pre-breeding efforts
- Raise awareness with key partners on the importance of *Musa* genetic resources conservation, documentation, exchange and sharing the benefits arising from their use
- Set priorities for research, breeding, and use of *Musa* genetic resources, ensuring critical links with the regional networks.

MusaNet members are from a wide range of *Musa* genetic resources interests. Membership is on an expertise basis and not on institutional or country representation basis. The networking structure of MusaNet consists of a Coordinating Secretariat, an Expert Committee and five Thematic Groups (Conservation, Diversity, Evaluation, Information and Genomics) in which experts discuss and propose solutions on critical thematic areas of *Musa* genetic resources. Critical links with the 4 Regional Research Networks and other key initiatives such as ProMusa are ensured with representation in the Expert Committee.

For more information: www.musanet.org

REGIONAL BANANA RESEARCH AND DEVELOPMENT NETWORKS

Regional banana research and development networks are important regional platforms for national programmes to agree on regional collaboration for *Musa* research and development and identify priorities to develop and implement projects.

There are currently 4 regional banana research networks:

- BAPNET - Banana Asia-Pacific Network
- BARNESA - Banana Research Network for Eastern and Southern Africa

- InnovatePlantain - Innovation Platform for Plantains in West and Central Africa (based at CARBAP), (*formerly Musaco*)
- MUSALAC - Plantain and Banana Research and Development Network for Latin America and the Caribbean

These networks are made up of national research organizations from all major banana-producing countries and provide coordination and support for regional research and development initiatives, including conservation efforts. The list of member countries for each network is given in ***Table 1***.

Table A.1. *Country members of each Regional Musa Research Network – updated August 2015*

	MUSALAC	InnovatePlantain	BARNESA	BAPNET
1	Argentina	Cameroon	Burundi	Australia
2	Bolivia	Congo, DRC	Congo, DRC	Bangladesh
3	Brazil	Congo, Republic of	Ethiopia	Cambodia
4	Colombia	Côte d'Ivoire	Kenya	China
5	Costa Rica	Gabon	Madagascar	India
6	Cuba	Ghana	Malawi	Indonesia
7	Dominican Republic	Guinea	Mozambique	Malaysia
8	Ecuador	Nigeria	Rwanda	Pacific Countries
9	Mexico		South Africa	Papua New Guinea
10	Nicaragua		Sudan	Philippines
11	Panama		Tanzania	Sri Lanka
12	Peru		Uganda	Taiwan
13	Puerto Rico			Thailand
14	Venezuela			Vietnam

Banana-producing countries have been represented in these networks to exchange perspectives, to propose regional research priorities and to mobilize resources around key activities. They function under the auspices of regional agricultural research fora and are coordinated by regionally-posted Bioversity scientists. Each network has a steering committee made up of a representative from every member country. Bioversity coordinators act mainly as facilitators and moderators for the networks. The steering committees have annual meetings hosted by participating countries. The network members collaborate through a suite of ongoing projects, workshops and training courses in the conservation, research and development of banana genetic resources in each region (including an *in situ* conservation project in East Africa). Over a period of 20 years, the networks' *modus operandi* has evolved considerably. They have become more objective-oriented, and they have broadened their area of action, from an early focus on production constraints (which nevertheless remain an important part of the agenda) to a broader concern with market-oriented development of the banana sector.

The regional networks play a crucial role in the implementation of the Strategy. To ensure full participation of regional networks in MusaNet, a member of each regional network is represented in the MusaNet EC and is present at the regional network steering committee meetings. Members of regional networks are also encouraged to participate in thematic groups based on expertise and to ensure a good regional coverage and representation.

For more information:

- MUSALAC: http://banana-networks.org/musalac/
- Innovate Plantain: http://banana-networks.org/innovate-plantain/

- BARNESA : http://banana-networks.org/barnesa/
- BAPNET : http://banana-networks.org/bapnet/

PROMUSA

ProMusa is a global network embracing a membership of researchers collaborating to address issues related to banana improvement, protection and production. The aim of ProMusa is to address global challenges faced by resource-poor smallholder banana farmers in developing countries collaboratively by bringing the expertise of scientists from diverse disciplines together and mobilizing the best science available. ProMusa is an information platform for (1) developing the global research and development agenda on banana, (2) facilitating and synergizing research efforts through the identification of opportunities for collaboration and funding exchanging information, (3) synthesizing research results into knowledge products and (4) delivering the outputs to various user groups.

The main activity is to promote and facilitate exchange of information, knowledge and know-how through the following:

- biennial scientific symposium (reported in proceedings)
- electronic newsletter
- online compendium of banana knowledge
- access to images, bibliographic information, and field/lab protocols and tools
- online discussion forum
- platform for community engagement
- mailing lists and contacts database

ProMusa is composed of three working groups on: (1) Production, (2) Protection and (3) Improvement. ProMusa does not directly implement or fund research activities. The primary and secondary target audiences are scientists and technicians from Research Centres and Universities with limited access to resources, students, development organizations and the local private sector.

ProMusa has about 500 members and almost all MusaNet meeting participants are members. Any interested person can subscribe online and take part in the working groups. MusaNet complements ProMusa in aspects of *Musa* genetic resources activities.

For more information: www.promusa.org

THE CGIAR RESEARCH PROGRAM ON ROOTS, TUBERS AND BANANAS - CRP-RTB

The CGIAR Research Program (CRP) on Roots, Tubers and Bananas (RTB) is one of a series of initiatives spearheaded by CGIAR to bring together the researchers and resources of multiple agricultural research-for-development centres to improve efficiencies, generate and harness synergies and increase impacts. RTB is now restructuring for its second phase due to begin in January 2017. The purpose of RTB is to tap the underutilized potential of root, tuber, and banana crops to improve food security, nutrition, and livelihoods. This programme has a component on conserving and accessing genetic resources and one on enhancing impact through partnerships with critical links to the Global *Musa* Strategy. The objectives of the CRP-RTB are to:

- Ensure that the *ex situ* conservation of RTB crops is efficient, relevant, and cost effective
- Strengthen and better understand *in situ* conservation and on-farm management towards resilient livelihoods.

- Improve the coverage of in-trust collections
- Stimulate the use of RTB germplasm through characterization, description of agronomic features, reaction to pests and diseases, abiotic stresses, nutritional and technological traits
- Promote the use of germplasm by facilitating access to information
- Strengthen the global system for the safe exchange of germplasm
- Proactively provide advocacy for the value of genetic resources to policy makers and donors.

CRP-RTB is considered to be vital to the implementation of a Global Strategy, both as a means for sharing the multidisciplinary characterization and for reaching consensus on joint actions to expand the coverage of collections or rationalize them. A key element in understanding the diversity held in collections, managing them efficiently and making the diversity available for use by breeders and other clients, is an efficient genetic resources information system. It draws on the strength of CGIAR centres and existing partnerships within a coherent programmatic framework and links genetic resources into a CRP-wide use framework. It also allows for cross-crop synergies, learning and linkages to regional research for development platforms.

For more information: www.rtb.cgiar.org

THE CGIAR RESEARCH PROGRAM ON GENEBANKS

The CGIAR Research Program on Genebanks is a partnership between the members of CGIAR Consortium and the GCDT. It is a programme for the management, as well as the secure and sustainable funding of plant genetic resource collections held by the 11 international genebanks members of the CGIAR Consortium. The objective is to conserve the diversity of plant genetic resources in CGIAR-held collections and to make this diversity available to breeders and researchers in a manner that meets high international scientific standards, is cost efficient, is secure, reliable and sustainable over the long-term and is supportive of and consistent with the ITPGRFA. CGIAR centres manage many of the largest and most important collections of crop diversity in the world. Centre collections serve as the foundation for their breeding and research and as the major source of genetic resources worldwide for plant breeders, as well as for researchers engaged in more basic biological research. CGIAR genebanks are major suppliers of material for (non-breeding) scientific research to CGIAR and other scientists. Such research, dependent upon the genebank collections, contributes to crop improvement and use and underpins considerable basic scientific research. Without reliable funding, even the best genebanks face a chronic inability to plan, to invest rationally, and to manage optimally.

For more information: http://www.cgiar.org/our-research/cgiar-research-programs/cgiar-research-program-for-managing-and-sustaining-crop-collections/

THE GLOBAL CROP DIVERSITY TRUST (GCDT)

In 2005, the newly established GCDT initiated a consultation process leading to the development of over 30 global crop and regional strategies for the *ex situ* conservation and utilization of crop diversity. The 2006 *Musa* Strategy was one of them. The strategies contribute to decision-making on the GCDT's allocation of resources. These strategies represent a major undertaking in the field of plant genetic resources, mobilizing experts to collaboratively plan for the more efficient and effective conservation and use of crop diversity. The process brought together collection managers, researchers, and other experts on plant genetic resources from developing and developed countries. The strategies focused on 8 main themes: regeneration, crop wild relatives, collecting, crop descriptors, information systems, user priorities, new technologies and research, and challenges to building a strategy for rational conservation. The priorities of the GCDT are for *ex situ* collections of the ITPGRFA and its Annex 1 crops, selected on the basis of their contribution to food security. *Musa* is an Annex 1 crop and one of the 22 priority crops for the Trust.

The strategies, although commissioned by the GCDT, were developed independently by the different communities involved, and will evolve as the situation of collections around the world changes.

For more information: www.croptrust.org

THE INTERNATIONAL TREATY ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE - ITPGRFA

As a key component of the ITPGRFA, the Multilateral System of access and benefit sharing (MLS) and its instrument, the Standard Material Agreement (SMTA) provide a common set of rules specified in a contractual format. This focuses on the facilitated access rules applying to individual transfers of those samples for certain purposes, namely utilization and conservation for research, breeding and training for food and agriculture. The SMTA recognises the need for international collective action to manage a common resource, PGRFA, in a manner that is more beneficial and efficient to everyone than any individual action would be. There are many non-monetary benefits from implementing the Treaty such as facilitated access to genetic resources as the major benefit, safeguarding, exchange of information, access to and transfer of technology, capacity-building, and also the sharing of monetary and other benefits of commercialization such as the social and environmental benefits. Facilitated access to these PGRFA is in itself the major benefit, making it possible for farmers and plant breeders to access the widest possible range of resources crucial for world food security. The principle aim of the benefit-sharing arrangements is to improve the conservation of, and the potential to sustainably use, plant genetic resources for food and agriculture, particularly for the benefit of farmers in developing countries and countries with economies in transition. Through the realization of those mechanisms, the Treaty could potentially contribute to rebalancing the *ex situ* conservation focus towards use and *in situ* conservation.

From a technical point of view, implementing the MLS at the national level requires the following actions: Identifying material under State control and management and in the public domain, using associated SMTAs, and defining signatory responsibilities. Other measures include creating a legal space for the Treaty in national access and benefit sharing (ABS) legislation and encouraging legal and natural persons to include material in the MLS. Some of the constraints impacting on the implementation of the treaty include the lack of appreciation of national dependence on foreign germplasm, negative perceptions about germplasm exchange because of claims of biopiracy and that the MLS would not benefit the country because lack of capacity for utilizing the resources. There are uncertainties concerning the concepts of ABS mechanisms among the scientific community, so the benefits involved in the MLS are not clearly perceived. There are misconceptions in relation to monetary benefits and a lack of clarity regarding both how benefits will accrue to farmers as custodians of agro-biodiversity and what links can be made with IPRs policies. The main political (collective action) challenges are: (1) coordination of different governance levels, (2) management of diverging interests and expectations within the PGRFA community, and (3) hierarchy between several global challenges such as genetic erosion and biodiversity loss; food security; rural poverty of small-holder farmers; crop adaptation to climate change; and bottom-up approach to development policy in agriculture.

The main contributions from a crop network such as MusaNet to the MLS are the following:

- Conservation: representativeness/completeness, security, efficiency of resource use, sustainability and responsiveness to global or regional threats
- Availability: proportion of conserved material available, extent to which available to all users, completeness of information system and accessibility of information to users and exchange of information
- Utilization: capacity for pre-breeding and breeding and collaboration on crop improvement and use programmes.

For more information: www.planttreaty.org

BOTANIC GARDENS CONSERVATION INTERNATIONAL - BGCI

BGCI is an international organization to ensure the world-wide conservation of threatened plants. BGCI represents over 700 members, mostly botanic gardens, in 118 countries. BGCI support members and the wider conservation community so that knowledge and expertise can be applied to reversing the threat of extinction facing one-third of all plants. BGCI supports the development and implementation of global policy, specifically the GSPC, at a global, regional, national, and local level.

BGCI works through the secretariat in London and regional offices in Kenya, the USA, Singapore and China to deliver the objectives of the GSPC. They produce publications, organize international meetings such as the World Botanic Gardens Conservation Congresses taking place every 4 years, and develop direct conservation programmes. BGCI also links very closely to the IUCN and the development of red list of threatened species. BGCI developed the Global Strategy for Plant Conservation with the objectives of understanding, documenting and using plant diversity, promoting education and awareness about plant diversity. These objectives are achieved through 16 specific targets including an assessment of the conservation status of all known plant species, as far as possible, to guide conservation action (target 2); developing and sharing information, research and associated outputs, and methods necessary to implement the Strategy (target 3); building capacity for the conservation of plant diversity (target 14) and establishing or strengthening institutions, networks and partnerships for plants conservation at national, regional and international levels to achieve the targets of this Strategy (target 16).

For more information: www.bgci.org

NATIONAL, REGIONAL AND INTERNATIONAL RESEARCH INSTITUTES

There are several institutes key in the Global Strategy by their activities of researching and maintaining *Musa* genetic diversity. Table 4 list the institutes that participated in the survey.

Table A.2. *Institutes maintaining Musa diversity and participated in the survey*

	Country	Acronym	Full Name of Institute
1	Global	ITC	International Transit Centre (ITC), Bioversity International
2	Global	IITA-NI	International Institute of Tropical Agriculture (IITA), Ibadan
3	Global	IITA-UG	International Institute of Tropical Agriculture (IITA), Uganda
4	Australia	DAFF-Mar	Department of Agriculture , Forestry and Fisheries, Maroochy Research Facility (DAFF-Mar)
5	Australia	DAFF-SJ	Department of Agriculture , Forestry and Fisheries, South Johnstone Research Facility (DAFF-SJ)
6	Brazil	EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
7	Burundi	IRAZ	Institut de recherches agronomiques et zootechniques (IRAZ)
8	Cameroon	CARBAP	Centre Africain de Recherche sur Bananiers et Plantains (CARBAP)
9	China	IFTR-GDDAS	Institute of Fruit Tree Research (IFTR), Guangdong Academy of Agricultural Sciences (GDAAS)
10	China	TBRI	Taiwan Banana Research Institute (TBRI)
11	China	TSFR Lab	Tropical and Subtropical Fruit Research Lab (TSFR)

12	Colombia	CORPOICA	Corporación Colombiana de Investigación Agropecuaria (CORPOICA)
13	Colombia	FEDEPLATANO	Federacion nacional de Plataneros de Colombia (FEDEPLATANO)
14	Congo DRC	FSK	Faculty of Sciences, University of Kisangani (FSK)
15	Congo DRC	INERA	Institut National pour l'Etude et la Recherche Agronomiques (INERA)
16	Cook islands	MoA	Ministry of Agriculture (MoA)
17	Costa Rica	CORBANA	Corporación bananera Nacional (CORBANA)
18	Côte d'Ivoire	CNRA	Centre National de Recherche Agronomique (CNRA)
19	Cuba	INIVIT	Instituto de Investigaciones de Viandas Tropicales (INIVIT)
20	Ethiopia	EIAR-Jimma	Ethiopia Institute of Agricultural Research (EIAR), Melkassa Research Center
21	Ethiopia	EIAR-Melkassa	Ethiopia Institute of Agricultural Research (EIAR), Jimma Research Center
22	Fiji	Sigatoka	Sigatoka Research Station (Sigatoka)
23	Fiji	SPC	Secretariat of the Pacific Community (SPC)
24	French Polynesia	SDR-FPNC	Service du développement rural, French Polynesia national collection (SDR-FPNC)
25	French Polynesia	SDR-PRFC	Service du développement rural, Pacific regional field collection (SDR-PRFC)
26	Gabon	IRAF	Institute de Recherches Agronomiques et Forestieres
27	France (Guadeloupe)	CIRAD	Centre de coopération internationale en recherche agronomique pour le développement (CIRAD)
28	India	KAU	Banana Research Station, Kerala Agricultural University (KAU)
29	India	NBPGR	National Bureau of Plant Genetic Resources (NBPGR)
30	India	NRCB	National Research Centre for Banana (NRCB)
31	Indonesia	ITFRI	Indonesian Tropical Fruit Research Institute (ITFRI)
32	Indonesia	IIS-RCB	Research Center for Biology, Indonesian Institute of Sciences, Research Center for Biology (IIS-RCB)
33	Indonesia	IIS-PBG	Indonesian Institute of Sciences, Purwodadi Botanic Garden (IIS-PBG)
34	Kenya	KARI-KISII	Kenya Agricultura Research Institute, Kisii (KARI-KISII)
35	Kenya	KARI-Thika	Kenya Agricultura Research Institute, Thika (KARI-THIKA)
36	Malawi	BARS	Bvumbwe Agricultural Research Station (BARS)
37	Malaysia	MARDI	Malaysian Agricultural Research and Development Institute (MARDI)
38	Mauritius	AREU	Agricultural Research and Extension Unit (AREU)
39	Mexico	CICY	Centro de Investigacion Cientifica de Yucatan (CICY)

40	Mexico	INIFAP	Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)
41	Myanmar	DAR	Department of Agricultural Research
42	Papua New Guinea	NARI	National Agricultural Research Institute
43	Philippines	BPI	Bureau of Plant Industry (BPI)
44	Philippines	UPLB	University of the Philippines, Institute of Plant Breeding (UPLB)
45	Puerto Rico	USDA	United State Depart. Of Agriculrure, Tropical Agriculture Research Station (USDA-ARS)
46	Rwanda	ISAR	Rwanda Agriculture Board, formerly Institut des Sciences Agronomiques du Rwanda, (ISAR)
47	Samoa	MAF	Ministry of Agriculture and Fisheries (MAF)
48	South Africa	ARC-ITSC	ARC-Institute for Tropical and Sub tropical Crops of the Agricultural Research Council (ARC-ITSC)
49	Sri Lanka	HORDI	Horticultural Crops Research and Development Institute (HORDI)
50	Sudan	ARC	Agricultural Research Corporation (ARC)
51	Tanzania	ARI-Maruku	Agricultural Research Institute Maruku (ARI-MARUKU)
52	Togo	ITRA	Institut togolais de recherche agronomique (ITRA)
53	Uganda	NARO	National Agricultural Research Organisation (NARO)
54	USA	Waimea	Waimea Valley Arboretum and Botanical Garden
55	Vanuatu	VARTC	Vanuatu Agricultural Research and Technical Centre (VARTC)
56	Vietnam	FAVRI	Fruit and Vegetable Research Institute (FAVRI)

ANNEX B.
THE 2016 STRATEGY DEVELOPMENT PROCESS

The 2016 Global *Musa* Strategy builds on the efforts and contributions of the many experts consulted in the development of the first Global Strategy for the Conservation of *Musa* (Banana), which was developed between 2004 and 2006 in consultation with a large number of individuals.

Stakeholders, including donors, partners, and beneficiaries, have provided extensive input into the development of the 2006 Strategy. Several institutes contributed by providing detailed information on the status of urgent needs of their *ex situ* collections. A background document on *Musa* diversity, origins and conservation needs was prepared by Prof. Edmond De Langhe. In addition, visits were made by taxonomic experts to collections in Kenya, Papua New Guinea, Rwanda and Vietnam. A number of consultation meetings were held to analyse survey results and define major elements of the Strategy. The broad conservation needs at different levels (international, regional, national, and local) and priorities were identified with major stakeholders. Discussions were also held during Bioversity's regional *Musa* research networks' meetings representing national agricultural research institutes (NARIs) in Sub-Saharan Africa, Asia and the Pacific. A draft Strategy document was regularly discussed with the scientific staff of the GCDT. Based on all feedback obtained, the first Strategy was finalized in early 2006 and was subsequently published on the Bioversity, Trust, Food and Agriculture Organization of the United Nations (FAO) and other websites. The GCDT used the 2006 Strategy to allocate funding to priority activities identified in the strategy. The Taxonomic Advisory Group (TAG) was established in 2006 to provide guidance for the implementation of the Strategy. TAG met in 2006 in Cameroon and in 2008 in India to discuss the implementation of the Global Strategy and make a number of recommendations.

There was a need to review and update the 2006 Strategy and strengthen the component on utilization to ensure that stakeholders can benefit from the secured conservation and maximize the use of genetic resources in improvement and other research programmes. The proposal to create the Global *Musa* Genetic Resources Network (MusaNet) was presented as the means to coordinate the implementation of a revised Global Strategy.

A number of follow-up discussions and consultations arose from the MusaNet March 2011 meeting which led to this revised Global Strategy. The MusaNet EC (including the Regional Banana Research Networks, GMGC and ProMusa) regularly discusses priorities and progress during conference calls and organised 16 calls from September 2011 to June in 2013.

Each of the four TGs (at that time GMGC had not yet merged with MusaNet as the Genomics Thematic Group) agreed on priorities and needs and developed a specific Thematic Strategic Plan. These Plans include a description of:

- Thematic Strategy Outputs
- Major issues, challenges to be addressed, and urgent needs and gaps based on the current situation
- Key users, stakeholders, and target groups
- What will be achieved in broad terms (expected outputs/products/results)
- Priorities and recommendations for actions.

These Thematic Plans form the basis for the Chapters' sections – Where we want to go.

In 2011, the newly reformed CGIAR approved a number of Research Programs aligning the research of its 15 Research Centres and their partners into multidisciplinary programs. Support to the conservation and use of *Musa* genetic resources and its partnership including MusaNet is mainly provided through the

CGIAR Research Program (CRP) on Roots, Tubers and Bananas (CRP-RTB) and support is provided to the international collection at the ITC through the CRP providing support to the CGIAR genebanks.

In order to update the information on the current status of the *Musa* collections worldwide, a global survey was carried out from September 2012 to May 2015 (*see Annex D. MusaNet Global Survey of ex situ collections – 2012-2015*). Over 60 collections were contacted with the support of the Regional *Musa* Research Networks and the MusaNet Secretariat to provide updated information on the following:

- Information on the Institute and Curator of collection
- Content of the *Musa* Collection
- Germplasm Management
- Documentation and Information
- Germplasm Exchange and Dissemination
- Long-term Security of the Collection.

By September 2013, 54 collections participated in this survey (*see Annex D. List of Musa Collections that participated in the 2012-2014 survey*). The information from the surveys formed the basis for the Chapter 2 Current Status of *Musa* Conservation and Use - Where we are today and guides the direction of the Strategy based on the current needs and opportunities.

In October 2013, an advanced draft document was sent out to all MusaNet members for a first review. In November 2013, a version based on the feedback of MusaNet members was circulated widely before finalization the MusaNet consultation in Guadeloupe in December 2013. Based on feedback received and many discussions, the structure of the Strategy was revised to have each Chapter include the following main sections:

- Where we are – status
- Where we want to go – strategy
- How we will get there – plan of action.

And the Strategy was divided into 4 main parts are follows:

- Introduction to the Global *Musa* Strategy
- PART A-PLANT DIVERSITY - Understanding *Musa* genetic diversity and domestication process. Wild relatives, edible AA and triploids
- PART B – IDENTIFICATION – Taxonomy and Characterization
- PART C – MANAGEMENT – *Ex situ* and *In situ*/On-farm conservation and the Global *Musa* Collection ITC
- PART D – USE - Evaluation, documentation and sharing information, distribution and safe exchange genetic and improvement.
- SUMMARY OF ACTIONS: from all PARTS A-B-C-D
- ANNEXES – References, Acronyms, Glossary, Tables and Figures etc.

The Strategy development in brief:

- 2005-2006: Development of the First Global *Musa* Strategy under the guidance of the Trust and led by INIBAP
- June 2006: First meeting of the TAG, Cameroon
- October 2008: Second meeting of the TAG, India

- 2011: CRP-RTB approved
- March 2011: Establishment of MusaNet
- September – December 2011: Establishment of MusaNet EC and 4 TGs
- January to June 2012: Development of the 4 TGs Strategic and Work Plans
- July 2012: MusaNet/Trust joint workshop to discuss the Effective Use of Genetic Diversity for Addressing Emerging Challenges in Banana and Plantain Breeding, Bogor, Indonesia.
- September 2012 to May 2013: Global Survey of all *Musa* Germplasm Collection Worldwide for updated information on current status of conservation and use
- September to December 2013: Development of the revised Global Strategy
- December 2013: MusaNet consultation in Guadeloupe
- January 2014 to April 2014: proposed restructuration of the draft Strategy document based on MusaNet EC feedback and discussions
- December 2014: MusaNet consultation in India
- April 2014 to December 2015 – drafting of individual Chapters
- January 2016-February 2016 – review by all MusaNet members
- February 2016 – August 2016 – finalization and publication.

ANNEX C. ACKNOWLEDGEMENT FOR THE 2006 GLOBAL CONSERVATION STRATEGY

The 2006 strategy has been developed through the synthesis of the ideas and information of a large number of individuals. Numerous members of staff of the International Network for the Improvement of Banana and Plantain (INIBAP) provided input throughout the strategy development process.

Edmond de Langhe (founding Director of INIBAP and now a private citizen) provided the expertise on the taxonomy of *Musa* origins of cultivated forms on which the strategy is founded, as well as many important perspectives on how a global strategy should take shape. Many collection curators dedicated significant time and effort to complete the detailed conservation survey. Rony Swennen (KUL, Belgium), Jeff Daniells (QDPI&F, Australia), Michael Pillay (IITA-Uganda) and Abdou Tenkouano (IITA-Cameroon), as well as Deborah Karamura (INIBAP-Uganda) deserve special mention for their individual contribution.

Eldad Karamura (INIBAP-Uganda) and Deborah Karamura and Agustin Molina (INIBAP-Philippines) organized the hosting of discussions for Sub-Saharan Africa and for Asia and the Pacific, respectively, at regional network steering committee meetings. Regional network steering committee members contributed valuably to discussions. For the Asia and the Pacific region Jeff Daniells, Sathiamoorthy (NRCB, India), Suzanne Sharrock (BGCI, United Kingdom) and Percy Sajise (Bioversity-Malaysia) took part in an evaluation committee which involved analysing information and the evaluations of the collections and providing their considered opinions on which collections should be priority for support.

The staff of *Katholieke Universiteit Leuven* and the *Universiteitsbibliotheek Leuven*, hosted the exhibition on banana diversity 'No end to the banana', which provided the venue for launching the Global Conservation Strategy for *Musa*; Rony Swennen, Kodjo Tomekpe (CARBAP, Cameroon), Edmond de Langhe, Richard Markham (INIBAP and Bioversity -France) and Cary Fowler (Trust, Italy) gave presentations or speeches in support of the Strategy at this event.

Ana Beretta and Campbell Davidson coordinated the attendance of Elizabeth Arnaud (INIBAP) in the meeting of the Regional Strategy for the Americas.

Charlotte Lusty (INIBAP) coordinated the collecting of input and feedback from stakeholders and took the lead in drafting this strategy document.

Finally, Brigitte Laliberté and Jane Toll of the Global Crop Diversity Trust have both been generous with their time in providing feedback on the strategy and helping to align it with the Trust's thinking.

ANNEX D.
MUSANET GLOBAL SURVEY OF *EX SITU* COLLECTIONS – 2012-2015

GLOBAL *MUSA* SURVEY TEMPLATE: A GLOBAL SURVEY OF *MUSA* COLLECTIONS AROUND THE WORLD

Introduction letter

In 2006, a first Global Strategy for the Conservation of *Musa* Genetic Resources was developed based on feedback from several collection curators and many other scientists working in this area. The strategy facilitated the submission of several projects on improving the conservation and use of *Musa*. It also stimulated the establishment of MusaNet, the Global *Musa* Genetic Resources Network. Now 9 years later, MusaNet is updating the Global Strategy and the information on the key *Musa* collections around the world to increase support and funding to crucial collaborative actions.

As a curator of a *Musa* germplasm collection, we kindly request you to support this effort by completing/updating this questionnaire. We are particularly interested in any updated information on the following:

- Section A: Information on the Institute and Curator of collection
- Section B: Content of the *Musa* Collection
- Section C: Germplasm Management
- Section D: Documentation and Information
- Section E: Germplasm Exchange and Dissemination
- Section F: Long-term Security of the Collection

The survey has 49 questions and will take you approximately 30 minutes to finish. The feedback received will contribute to updating the MusaNet Global Strategy for the Conservation and Utilization of *Musa* Genetic Resources. Please note that if your institute no longer maintains a collection of *Musa* sp, please kindly send us an email to inform us. We would be grateful for this important information and update our database.

Your participation in the development of this initiative is highly valued. By fill in this survey you will contribute to the updating of the Global Strategy. If you are interested in taking part in its review before it is finalized, please let us know (*see question 49*) and we will make sure you receive a draft copy for your feedback.

Table A.1. *Country members of each Regional Musa Research Network – updated August 2015*

Section A: Information on the Institute and Curator	
1. Name and address of institution where the *Musa* collection is:	
Name of institution	
Address	
Country	
2. Curator managing the *Musa* collection:	
Last name	
First name	
Address	

Email	
Phone number	
3. Name of person completing this survey if not the same as 2 above:	
Last name	
First name	
Position	
Institute	
Address	
Email:	

4. Please indicate (with an X in the left column) the type of the institute maintaining the *Musa* collection:	
	Public-funded institute (government institute, university, public-funded research institute)
	Private institute
	Other, please specify:

5. Please indicate (with an X in the right columns) what responsibility the institute has in maintaining the *Musa* collection.	**Yes**	**No**	**Don't know**
Your institute owns the collection			
Your institute is officially mandated by your government for the conservation of *Musa*			
Your institute conducts research on *Musa*			
Your institute provides most of the funds for the *Musa* collection. If the answer is "No", please specify the origin of funds:			

6. If the institute is responsible for the conservation of *Musa*, please specify at what level:	
Collection objective:	**Indicate with X**
National (*mainly for local or national conservation, distribution and use*)	
Regional (*for conservation, and distribution at the regional level, e.g. Americas, Asia, Africa etc*)	
Global (*mainly for international conservation, distribution and use*)	
Other: please specify (*eg. only for the use of a specific institute`s breeding programme*)	

7. If you have answered "no" to the second sub-question of question 5, please specify, when there is one, the responsible institute for *Musa* germplasm conservation in your country *(providing contact details if possible)*:

8. Please specify the responsibility of the institute vis a vis the management of the *Musa* collections.	
	Yes, our institute coordinates the management of several *Musa* collections in the country.
	No, our institute is only responsible for the specific collection mentioned in no 1.
	Other, please specify:
	If YES, please list the stations that are included in this survey:

9. Indicate the year the collection was established:	
Year	
	Please include any useful comment regarding the establishment of the collection.

Section B: Content of the *Musa* Collection

IMPORTANT: If you are providing information on more than one collection, please copy and paste the relevant tables below and indicate at the top the name of the collection for which you are providing information.

10. Please rank the uses/functions of the *Musa* collection (indicate with X):

Collection objective:	First priority	Second priority	Third priority
Genetic resources conservation			
Characterization and genetic studies			
Support to breeding programmes			
Dissemination/distribution			
Other: please specify			

11. Please indicate the number of accessions in the collection:

Type of materials	No of accessions				
	Field collection	Tissue culture/ *in vitro*	Greenhouse/ nursery	Cryopreservation	Other Please specify
Total no. of accessions in the collection					
Wild taxa					
Cultivars					
Breeding lines					
Other - please specify:					

12. If you have wild taxa (species and sub-species) in your collection, please list the name and number of accessions (please add rows as needed):

Wild taxa	No of accessions
Species 1 – *please enter the taxonomic name*	
Species 2 – *please enter the taxonomic name*	
Species 3 – *please enter the taxonomic name*	
Species 4 – *please enter the taxonomic name*	
Species 5 – *please enter the taxonomic name*	
Add as many lines as needed here	

13. Please estimate the number of accessions in the following categories:

Collected from your country's wild areas	
Coming from local farmers	
Coming from your national breeding programme	
Introduced (not native to your region or country)	
Origin not known	
Other – please specify:	

14. Do you have an agreement with another institute for keeping a safety-duplication of another *Musa* collection?

Yes		please indicate the institute/country:	
No			

If YES, please indicate (with an X) in which form is the safety-duplication:

	Field genebank
	In vitro conservation
	Cryo-preservation

15. Indicate the changes of the collection over the last decade:		
Change	**No.**	**How?**
How many accessions have been acquired in the past 10 years?		
How many accessions have been lost from the collection in the past 10 years?		
How many accessions have been removed as they were duplicates/synonyms?		
How many accessions have been removed to focus on the institute's priority materials?		
Other – please specify:		

16. What part of the collection (which accessions) make the collection important or unique? Please describe their uniqueness:

17. Please describe any gaps in the collection in terms of genetic diversity that would help fulfil your conservation, research or breeding objectives. Indicate the nature of the gaps (species, geographic, traits):

18. Is there any genetic diversity in your country, either in cultivation or in the wild, that is poorly represented in *ex situ* collections? If yes, please specify the diversity missing.

19. Please describe any RECENT activities to collect germplasm (*species and area*):

20. Please describe any FUTURE PLANS to collect germplasm (*species and area*):

Section C: Germplasm Management and Use

21. If you maintain a FIELD collection, please describe the size and planting:	
Number of plants per accession:	
Space between each plant of the same accession (*in metres*):	
Total size of field collection (*in hectare*):	
Space between 2 different accessions (*in metres*):	

22. If you maintain an IN VITRO collection, please indicate the following:	
Number of replicates per accession:	
Storage duration between subcultures (*in months*):	
Is the material is stored under normal growth conditions or slow growth conditions?	
Indicate the storage temperature (*in °C*):	
Specify the light conditions, i.e. light regime for the photoperiod, (*e.g. 12h:12h light/dark*):	
Specify the light intensity:	
Constraints regarding facilities (*e.g. continuous power supply, access to a power generator, availability of a separate room with appropriate storage conditions, etc*):	
Constraints regarding plant materials (*e.g. genotypes not adapted to the storage conditions, protocol not optimized, contamination etc.*):	
Others – please indicate any additional information you think might be of interest:	

23. What clonal propagation material is used for distribution?			
Methods	**Mostly**	**Rarely**	**Never**
Suckers			
Corms			
In vitro plantlets			
Other (Please specify)			

24. What facilities are available (tick as many as appropriate)?			
Facilities	**Yes**	**Yes, provided by partner institute**	**No**
Multiplication facilities (nursery, etc.)			
Multiplication facilities - *in vitro* lab			
Laboratory characterization facilities (for genotype validation)			
Irrigation facilities			
Post-entry quarantine			
Virus-indexing			
Cryopreservation facilities			
Other – specify:			

25. If you have VIRUS-INDEXING facilities, please indicate the services and methods (with an X):	
	Symptoms
	ELISA
	PCR
	Other: please specify:

26. Which are the most damaging conditions affecting your field collection (indicate with an X)?			
Biotic conditions	**Present, major effect**	**Present, but minor effect**	**Not present**
Fusarium wilt (indicate which race and/or VCG, if known)			
BBrMV - Banana Bract Mosaic Virus			
BBTV - Banana Bunchy Top Virus			
BSV- Banana Streak Virus			
CMV - Cucumber Mosaic Virus			
Bacterial wilt (indicate which one, causal pathogen)			

Black leaf streak			
Other Mycosphaerella leaf spots			
Nematodes (indicate genus/species, if known)			
Weevils			
Others biotic conditions - Please specify:			

Abiotic conditions	Present, major effect	Present, but minor effect	Not present
Drought			
Flooding			
Cold			
Heat			
Wind			
Need anything on soil conditions? (salt?)			
Rummaging animals?			
Other abiotic conditions – please specify:			

27. How is the field collection managed? (X wherever appropriate)				
	Regularly	Occasionally	Rarely	Never
Herbicides				
Pesticides				
Fungicides				
Weed eradication (manual)				
Cover crop (renewed)				
Mulching (other than banana leaves)				
Fertilizer				
Compost				
Irrigation				
Desuckering				

28. What is the frequency of replanting the field collection?			
Frequency of replanting	Every year	Every 2 years	Less frequent than every 2 years
Transplanting less than 10% of the collection			
Transplanting between 10-50% of the collection			
Transplanting more than 50% of the collection			

29. Please describe the safety-duplication situation of your collection by indicating an estimate percentage (%):	
% safety duplicated at ITC (International Transit Centre, Belgium)	% of total accessions
% safety duplicated *in vitro* elsewhere in your country	
% safety duplicated in field collections elsewhere in your country	
% safety duplicated in vitro elsewhere in another country (not including ITC)	
% safety duplicated in field collections elsewhere in another country	

30. If less than half of the collection is safely duplicated, please indicate the main reasons why and if there are plans to safely duplicate 100% of the collection?

31. Indicate the activities carried out concerning the collection (only one X per row):			
Activity	Carried out on a routine basis	Carried out occasionally	Not carried out
Characterization for taxonomic traits (flower, fruits, etc)			
Characterization using molecular markers			
Evaluation of host reaction to pests and diseases			
Evaluation of other important traits			
Breeding (hybrid or clonal trials)			

32. Do you have an operation manual containing standard procedures and protocols for the management activities of the collections mentioned above? Please indicate with an X	
	Yes
	No

Section D: Documentation and Information

33. Describe the management of the data on the germplasm collection (tick as many boxes as apply):				
	Spreadsheet (e.g. Excel)	Database (E.g Access)	Paper-print	Not collected
Passport data (name, origin, etc.)				
Photographs of accessions				
Characterization data				
Evaluation data				
Management data				
Shipment data of germplasm				

34. If the data are managed with a database management system, please provide the name of the software(s) used *(commercial system or developed by your institute or another)*:

35. Describe if a new data management system is being considered in the near future and specify which one:

36. Describe the access to and availability of the information (indicate with an X):		
	Carried out on a routine basis	Carried out occasionally
Data publically available to all		
Data available as a published catalogue		
Data available upon request as electronic downloads		
Data available on our institute website at all times		
Other, please specify:		

37. Describe the links between your collection and the *Musa* Germplasm Information System (MGIS) managed by Bioversity International (indicate with an X):	
Our list of accessions is included in MGIS and regular updates are sent to Bioversity	
We provided a list of accessions a long time ago and is currently outdated in MGIS	
We do not provide data to MGIS yet	
I am not familiar with MGIS	
Other, please specify:	

38. Please describe any plans, needs or constraints concerning managing information on accessions in the collection

Section E: Germplasm Exchange and Dissemination

39. Is the *Musa* collection available for distribution outside of your country?

	Yes
	No

If Yes, what type of agreement is used with the germplasm distributed? Indicate with an X

	No Material Transfer Agreement (MTA) is used
	An MTA developed by our institute is used for bilateral agreements
	The Standard MTA of the International Treaty
	Other – please specify:

40. What are the conditions that apply to requests for germplasm (e.g. *formal or informal approval processes, agreements or any other requirements relating to the availability of germplasm*)?

41. How frequently are samples of germplasm distributed from the collection (one tick per row)?

	Once or more every month	Less than once a month - *but at least once a year*	Less than once a year
To users within the institute			
To local users – near the institute			
To distant users in the country			
To users outside the region			

42. What is the average number of accessions distributed per year?	
To local users	
To more distant users in the country	
To users outside the country but within the region	
To users outside the region	
Other, please specify:	

43. Please indicate with an X, the main groups of external users of your *Musa* collection:			
Users	**Frequent use**	**Rare use**	**Never used**
Farmers			
Researchers			
Breeders			
NGOs			
Production labs			
Private sector			
Public sector			
Other, please specify:			

44. Indicate (with an X) the purpose for the use of the disseminated materials:			
Purposes	**Frequent use**	**Rare use**	**Never used**
Research activities			
Characterization			
Evaluation			
Pre-breeding			
Breeding			
Biotechnological research			
Distribution to growers			
Other, please specify:			

45. What factors, if any, limit the distribution and use of materials?			
Factors	**Yes, major factor**	**Yes, but minor factor**	**No effect**
Legal restrictions/government policy			
Phytosanitary regulations (permits/ certificates)			
Material is not certified disease free			
Limited quantity of material available			
Resources to prepare and ship germplasm			
Poor documentation status of germplasm			
Facilities /expertise to index the health status of germplasm			
Other, please specify:			

Section F: Long-term Security of the Collection

NOTE: The responses to the following questions are important in providing baseline information to estimate the costs of supporting *Musa* conservation at a global level.

46. What is the current status of the collection with respect to the following factors?				
Factors	**Very Good**	**Adequate**	**Inadequate**	**No opinion**
Funding for routine operations and maintenance				
Number of trained staff				
Status of buildings, facilities and equipment				
Funding for collecting germplasm				
Funding for research on the collection				
Level of use by breeders, researchers or growers				

47. Please describe the major needs or concerns influencing the long-term sustainability of your collection:

48. What changes to the present situation would you consider to be essential for the long-term conservation of *Musa* at a global level?

49. Please indicate (with an X) if you would like to participate in the revision of a draft of the updated Global *Musa* Strategy:	
	YES - I would like to provide feedback on a draft
	NO – but I would like to receive a copy of the final version

Thank you for completing this questionnaire

LIST OF MUSA COLLECTIONS THAT PARTICIPATED IN THE 2012-2015 GLOBAL SURVEY

Table D.1. *Collections that participated in the Global* Musa *Survey.*

	Country	Institute	Date received
1	Global	ITC	24/09/2012
2	Global	IITA, Nigeria	03/10/2012
3	Global	IITA, Uganda	02/11/2012
	Africa – Eastern and Southern		
4	**Ethiopia**	EIAR-Jimma	24/08/2012
5	Ethiopia	EIAR-Melkassa	03/09/2012
6	Kenya	KARI-KISII	01/02/2013
7	Kenya	KARI-Thika	18/09/2012
8	Malawi	BARS	13/09/2012
9	Mauritius	AREU	09/11/2012
10	South Africa	ARC-ITSC	07/09/2012
11	Sudan	ARC	09/09/2012
12	Tanzania	ARI Maruku	27/08/2012
13	Uganda	NARO	13/05/2013
	Africa - Western		
14	Burundi	IRAZ	12/09/2012
15	Cameroon	CARBAP	11/09/2012
16	Congo DRC	FSK	14/02/2013
17	Congo DRC	INERA	03/09/2012
18	Cote d'Ivoire	CNRA	12/04/2013
19	Gabon	IRAF	07/07/2015
20	Rwanda	ISAR	08/02/2013
21	Togo	ITRA	13/09/2012
	Latin America and Caribbean		
22	Brazil	EMBRAPA	08/02/2013
23	Colombia	CORPOICA	08/10/2012
24	Colombia	FEDEPLATANO	27/02/2013
25	Costa Rica	CORBANA	11/09/2012
26	Cuba	INIVIT	20/09/2012
27	France – Guadeloupe	CIRAD	15/02/2013
28	Mexico	CICY	13/04/2013
29	Mexico	INIFAP	27/08/2012
30	USA	USDA –Puerto Rico	18/09/2012
31	USA	Waimea	11/09/2012
	Asia and Pacific		
32	Australia	DAFF-Mar	10/09/2012
33	Australia	DAFF-SJ	21/08/2012
34	China	IFTR-GDAAS	02/09/2012
35	China	TBRI	14/09/2012
36	China	TSFR Lab	17/09/2012
37	Cook Islands	MoA	30/08/2012
38	Fiji	Sigatoka	28/08/2012
39	Fiji	SPC	24/08/2012
40	French Polynesia	SDR-FPNC	25/09/2012
41	French Polynesia	SDR-PRFC	25/09/2012

42	India	KAU	06/03/2013
43	India	NBPGR	31/01/2013
44	India	NRCB	20/09/2012
45	Indonesia	IIS-PBG	03/09/2012
46	Indonesia	IIS-RCB	07/09/2012
47	Indonesia	ITFRI	04/09/2012
48	Malaysia	MARDI	04/09/2012
49	Myanmar	DAR	12/10/2015
50	Papua New Guinea	NARI	20/03/2013
51	Philippines	BPI	11/09/2012
52	Philippines	UPLB	20/09/2012
53	Samoa	MAF	03/09/2012
54	Sri Lanka	HORDI	03/09/2012
55	Vanuatu	VARTC	19/10/2012
56	Vietnam	FAVRI	20/09/2012

GLOBAL *MUSA* SURVEY – DETAILS

Table D.2. *Institutes managing* Musa *genetic diversity – number of accessions.*

	Country	Institutes Acronym	Field collection	*In vitro* collection	Green-house	Cryopreser-vation
1	Global	ITC	0	1404	0	866
2	Global	IITA-Nigeria	371	180	0	0
3	Global	IITA-Uganda	217	0	0	0
		TOTALS	**588**	**1,584**	**0**	**866**
	Africa – Eastern and Southern					
4	**Ethiopia**	EIAR-Jimma	88	3	0	0
5	Ethiopia	EIAR-Melkassa	107	0	0	0
6	Kenya	KARI-KISII	166	0	0	0
7	Kenya	KARI-Thika	63	5	5	0
8	Malawi	BARS	70	0	0	0
9	Mauritius	AREU	56	10	10	0
10	South Africa	ARC-ITSC	99	24	0	0
11	Sudan	ARC	357	0	0	0
12	Tanzania	ARI-Maruku	260	0	0	0
13	Uganda	NARO	452	0	0	0
		TOTALS	**1,718**	**42**	**15**	**0**
	Africa - Western					
14	Burundi	IRAZ	298	155	0	0
15	Cameroon	CARBAP	650	0	0	0
16	Congo DRC	FSK	107	10	11	0
17	Congo DRC	INERA	68	0	0	0
18	Côte d'Ivoire	CNRA	58	0	0	0
19	Gabon	IRAF	115	0	0	0
20	Rwanda	ISAR	117	0	0	0
21	Togo	ITRA	13	0	0	0
		TOTALS	**1,426**	**165**	**11**	**0**
	Asia and Pacific					
22	Australia	DAFF-Mar	0	417	0	0
23	Australia	DAFF-SJ	200	0	0	0
24	China	IFTR-GAAS	314	215	65	0
25	China	TBRI	6	220	220	0
26	China	TSFR Lab	110	110	0	0
27	Cook islands	MoA	35	0	0	0
28	Fiji	Sigatoka	13	7	0	0
29	Fiji	SPC	0	185	0	0
30	French Polynesia	SDR-FPNC	178	15	0	0
31	French Polynesia	SDR-PRFC	22	26	0	0
32	India	KAU	300	20	0	0
33	India	NBPGR	0	415	0	50
34	India	NRCB	313	0	0	0
35	Indonesia	ITFRI	148	44	70	0
36	Indonesia	IIS-RCB	150	30	50	0
37	Indonesia	IIS-PBG	103	0	26	0
38	Myanmar	DAR	30	13	0	0

	Country	Institutes Acronym	Field collection	In vitro collection	Green-house	Cryopreser-vation
39	Papua New Guinea	NARI	217	24	0	0
40	Philippines	BPI	507	11	216	0
41	Philippines	UPLB	180	20	0	0
42	Samoa	MAF	30	16	0	0
43	Sri Lanka	HORDI	20	0	0	0
44	Vanuatu	VARTC	154	0	0	0
45	Vietnam	FAVRI	120	32	0	0
		TOTALS	**3,150**	**1,820**	**647**	**50**
	Latin America and Caribbean					
46	Brazil	EMBRAPA	400	250	0	0
47	Colombia	CORPOICA	188	164	0	0
48	Colombia	FEDEPLATANO	161	0	0	0
49	Costa Rica	CORBANA	120	15	0	0
50	Cuba	INIVIT	346	137	61	0
51	Guadeloupe	CIRAD	391	0	0	0
52	Malaysia	MARDI	200	180	100	0
53	Mexico	CICY	115	0	47	0
54	Mexico	INIFAP	37	0	0	0
55	Puerto Rico	USDA	170	150	0	10
56	USA	Waimea	41	0	17	0
		TOTALS	**2,169**	**896**	**225**	**10**
			9,051	**4,507**	**898**	**926**

Table D.3. *Year of establishment of the 56 surveyed* Musa *ex situ collections*

Country	Institute	Year
Congo DRC	INERA	1933
Guadeloupe	CIRAD	1950
China	TBRI	1967
Cameroon	CARBAP	1970
Ethiopia	EIAR-Jimma	1970
India	KAU	1970
Cuba	INIVIT	1971
Indonesia	IIS-PBG	1972
Philippines	BPI	1975
Brazil	EMBRAPA	1976
Australia	DAFF-SJ	1981
Malaysia	MARDI	1982
Burundi	IRAZ	1984
GLOBAL	ITC	1985
India	NBPGR	1986
Papua New Guinea	NARI	1986
Australia	DAFF-Mar	1988
China	IFTR-GDDAS	1989
Costa Rica	CORBANA	1989
Mexico	INIFAP	1990
Philippines	UPLB	1990
Samoa	MAF	1990
Puerto Rico	USDA	1992
South Africa	ARC-ITSC	1992
Ethiopia	EIAR-Melkassa	1993
USA	Waimea	1993
India	NRCB	1994
Vietnam	FAVRI	1994
Fiji	SPC	1995
Tanzania	ARI-Maruku	1995
Global	IITA-UG	1996
Kenya	KARI-KISII	1997
Kenya	KARI-Thika	1997
Côte d'Ivoire	CNRA	1998
Mauritius	AREU	1998
Mexico	CICY	1998
Malawi	BARS	1999
Rwanda	ISAR	1999
Cook islands	MoA	2000
China	TSFR Lab	2001
Indonesia	ITFRI	2002
Sudan	ARC	2003
Colombia	CORPOICA	2004
Congo DRC	FSK	2005
Fiji	Sigatoka	2005
Indonesia	IIS-RCB	2006

Country	Institute	Year
Australia	DAFF-Mar	1988
Australia	DAFF-SJ	1981
Brazil	EMBRAPA	1976
Burundi	IRAZ	1984
Cameroon	CARBAP	1970
China	IFTR-GDDAS	1989
China	TBRI	1967
China	TSFR Lab	2001
Colombia	CORPOICA	2004
Colombia	FEDEPLATANO	2008
Congo DRC	FSK	2005
Congo DRC	INERA	1933
Cook islands	MoA	2000
Costa Rica	CORBANA	1989
Côte d'Ivoire	CNRA	1998
Cuba	INIVIT	1971
Ethiopia	EIAR-Jimma	1970
Ethiopia	EIAR-Melkassa	1993
Fiji	Sigatoka	2005
Fiji	SPC	1995
French Polynesia	SDR-FPNC	2007
French Polynesia	SDR-PRFC	2007
Gabon	IRAF	2013
Global	IITA-NI	2007
Global	IITA-UG	1996
GLOBAL	ITC	1985
Guadeloupe	CIRAD	1950
India	KAU	1970
India	NBPGR	1986
India	NRCB	1994
Indonesia	IIS-PBG	1972
Indonesia	IIS-RCB	2006
Indonesia	ITFRI	2002
Kenya	KARI-KISII	1997
Kenya	KARI-Thika	1997
Malawi	BARS	1999
Malaysia	MARDI	1982
Mauritius	AREU	1998
Mexico	CICY	1998
Mexico	INIFAP	1990
Myanmar	DAR	2010
Papua New Guinea	NARI	1986
Philippines	BPI	1975
Philippines	UPLB	1990
Puerto Rico	USDA	1992
Rwanda	ISAR	1999

Country	Institute	Year
Global	IITA-NI	2007
French Polynesia	SDR-FPNC	2007
French Polynesia	SDR-PRFC	2007
Colombia	FEDEPLATANO	2008
Uganda	NARO	2008
Myanmar	DAR	2010
Sri Lanka	HORDI	2010
Vanuatu	VARTC	2010
Togo	ITRA	2012
Gabon	IRAF	2013

Country	Institute	Year
Samoa	MAF	1990
South Africa	ARC-ITSC	1992
Sri Lanka	HORDI	2010
Sudan	ARC	2003
Tanzania	ARI-Maruku	1995
Togo	ITRA	2012
Uganda	NARO	2008
USA	Waimea	1993
Vanuatu	VARTC	2010
Vietnam	FAVRI	1994

Taable D.4. *Number of accessions in the in vitro/tissue culture collections, in order of total size of collection.*

Country	*In vitro*	Wild taxa	Cultivars	Breeding lines	Others	Total
GLOBAL	ITC	212	1066	126		1,404
Australia	DAFF-Mar	85	289	43		417
India	NBPGR		200		215	415
Brazil	EMBRAPA	150		100		250
China	TBRI	7	197	6		220
China	IFTR-GDDAS	16	152	24	3	215
Fiji	SPC		164	21	164	185
Nigeria	IITA-NI					180
Malaysia	MARDI	85	78	10	17	180
Colombia	CORPOICA		149	8	7	164
Burundi	IRAZ					155
Puerto Rico	USDA					150
Cuba	INIVIT					137
China	TSFR Lab	16	59	12	23	110
Indonesia	ITFRI	4	37	3		44
Vietnam	FAVRI		32			32
Indonesia	IIS-RCB	10			10	30
French Polynesia	SDR-PRFC					26
Papua New Guinea	NARI					24
South Africa	ARC-ITSC	13	11			24
India	KAU		20			20
Philippines	UPLB	1			3	20
Samoa	MAF					16
Costa Rica	CORBANA		15			15
French Polynesia	SDR-FPNC					15
Philippines	BPI		11			11
Congo DRC	FSK		9	4		10
Mauritius	AREU		10			10
Fiji	Sigatoka					7
Kenya	KARI-Thika					5
Ethiopia	EIAR-Jimma					3
TOTAL	**31**	**599**	**2,479**	**357**	**442**	**4,494**

Table D.5. *Description of RECENT collecting activities (species and area).*

Country	Acronym	Description
China	IFTR-GDDAS	*M. acuminata* and *M. balbisiana*, and improved diploids in FHIA or EMBRAPA, etc.
China	TBRI	1. *Musa yamiensis* (Lanyu (Taitung), Taiwan). 2. *Ensete glaucum* (Pingtung, Taiwan). 3. *Musa balbisiana* (Chiayi, Taiwan).
China	TSFR Lab	We are going to collect some local cultivars when there is financial support.
Colombia	CORPOICA	Socialization in exhibitions with farmers. Interesting materials identification. Visits to farmers' lands that have interesting materials. Material collection and its introduction. Bananas and plantains at farmers' lands at Valle del Cauca.
Colombia	FEDEPLATANO	A banana that seems tolerant to Ralstonia strains, from Ariari zone (at east of Bogotá).
Cook islands	MoA	There are no planned recent activities to collect germplasm except to introduce other cultivars and accessions being held at CePaCT, SPC. These accessions are either from the Pacific region or elsewhere.
Côte d'Ivoire	CNRA	Collection of plantain accessions from local farmers' fields in bananas growing areas in the southern, western and eastern parts of the country. Morphological characterization of these collected accessions is on going.
Fiji	Sigatoka	Establishment of germplasm collection at Wainigata Research Station.
Fiji	SPC	In collaboration with Bioversity International and the Trust, SPC is coordinating a regional banana project which involves the collection of unique bananas from the Pacific countries have them virus tested, and send for establishment at French Polynesia, host of the Pacific banana collection. This collection will be characterised, evaluated and DNA fingerprinted. A core collection will be duplicated, distributed and conserved at international centres.
French Polynesia	SDR-PRFC	Promoting countries to duplicate cultivar in the regional collection through SPC.
Guadeloupe	CIRAD	Diploid *M. acuminata* cultivars from Comores islands were recently collected.
India	KAU	Exotic germplasm was obtained through NBPGR, New Delhi, NRCB, Trichy. In the process of regeneration and field establishment.
India	NRCB	Routine collections have been conducted in the areas of local diversity for edible diploids.
Indonesia	IIS-PBG	In early March of 2012, we have conducted two days of *Musa* exploration, collecting local banana cultivars from farmers in Pasuruan and Probolinggo regencies (East Java).
Indonesia	IIS-RCB	We collected *Musa acuminata* subsp banksii, *Musa lolodensis*, and *Musa schizocarpa* from Papua (Timika, Jayapura and Manokwari).
Indonesia	ITFRI	Triangle Mission in Manado and Halmahera (October 2012), target wild relatives and cultivars.
Kenya	KARI-Thika	MUSA expedition was carried out about 5 years in 3 major banana growing areas. 33 new cultivars were introduced into conservation site as a result of this exercise.
Malaysia	MARDI	At least 3 accessions from each of 14 states in Malaysia.
Mauritius	AREU	1. Field visit to a grower and collection of 2 accessions. 2. A customer of African origin wanted to introduce plantains for chip making. Since cultivars requested were not available at ITC, alternatives were selected from list of available in collection.
Philippines	BPI	1. Collection of pest and disease resistant cultivars. 2. Collection of cooking and dessert elite cultivars at local and regional level. 3. Field, screenhouse and in-vitro conservations of banana collections.
Philippines	UPLB	For the past 3 years (2009-2012) collecting missions have been carried out on local cultivars (cooking types) with short stature.
South Africa	ARC-ITSC	Received 24 genotypes from BIOVERSITY for panama, nematode and horticultural evaluations.
Tanzania	ARI-Maruku	Because there is no financial support, it happens by chance whilst on other activities to find a new cultivar and collect it.
Togo	ITRA	Since 2011 we have collected in the main banana area cultivation most of the banana species for multiplication and planting material for farmers according to regional project funded by WECARD/Banana.
Uganda	NARO	Landraces and East African diploids at border points between Uganda and Rwanda and Democratic Republic of Congo.
Vietnam	FAVRI	To survey and collect accessions in the Northern Mountainous Area.

Table D.6. *Most damaging abiotic conditions with major effects on field collections.*

Abiotic conditions	Collections having major effects	
Drought	Burundi-IRAZ	Mexico-CICY
	Cameroon-CARBAP	Nigeria-IITA
	Ethiopia-EIAR-Melkassa	Papua New Guinea-NARI
	Fiji-Sigatoka	Rwanda-ISAR
	India-NRCB	Samoa-MAF
	Indonesia-IIS-PBG	Uganda-IITA
	Indonesia-ITFRI	Uganda-NARO
	Kenya-KARI-Thika	USA-Waimea
	Mauritius-AREU	
Flooding	Fiji-Sigatoka	Samoa-MAF
	Mexico-INIFAP	USA-Waimea
	Nigeria-IITA	
	Papua New Guinea-NARI	
Cold	China-TSFR Lab	
	Malawi-BARS	
Heat	Ethiopia-EIAR-Melkassa	Mexico-CICY
	India-NRCB	Nigeria-IITA
	Indonesia-IIS-PBG	Papua New Guinea-NARI
	Indonesia-ITFRI	
	Malawi-BARS	
Wind	Burundi-IRAZ	Mauritius-AREU
	China-TBRI	Mexico-CICY
	China-TSFR Lab	Mexico-INIFAP
	Congo DRC-INERA	Nigeria-IITA
	Costa Rica-CORBANA	Papua New Guinea-NARI
	Ethiopia-EIAR-Melkassa	Philippines-UPLB
	Fiji-Sigatoka	Puerto Rico-USDA
	Guadeloupe-CIRAD	Samoa-MAF
	India-NRCB	Uganda-NARO
	Indonesia-IIS-PBG	USA-Waimea
	Kenya-KARI-KISII	
Poor soil conditions	India-NRCB	Tanzania-ARI-Maruku, salt injury
	Indonesia-IIS-PBG	Uganda-NARO-Low PH and soil fertility
	Nigeria-IITA - Poor soil	
Rummaging animals	Fiji-Sigatoka	Samoa-MAF
	Indonesia-IIS-PBG	USA-Waimea
	Kenya-KARI-Thika	
	Mexico-CICY	

Table D.7. *In vitro collections surveyed with number of accessions maintained.*

Country	Acronym	Total number of accessions
GLOBAL	ITC	1404
Australia	DAFF-Mar	417
India	NBPGR	415
Brazil	EMBRAPA	250
China	TBRI	220
China	IFTR-GDDAS	215
Fiji	SPC	185
Nigeria	IITA	180
Malaysia	MARDI	180
Colombia	CORPOICA	164
Burundi	IRAZ	155
Puerto Rico	USDA	150
Cuba	INIVIT	137
China	TSFR Lab	110
Indonesia	IIS-PBG	44
Vietnam	FAVRI	32
Indonesia	IIS-RCB	30
French Polynesia	SDR-PRFC	26
Papua New Guinea	NARI	24
South Africa	ARC-ITSC	24
India	KAU	20
Philippines	UPLB	20
Samoa	MAF	16
Costa Rica	CORBANA	15
French Polynesia	SDR-FPNC	15
Philippines	BPI	11
Congo DRC	FSK	10
Mauritius	AREU	10
Fiji	Sigatoka	7
Kenya	KARI-Thika	5
Ethiopia	EIAR-Jimma	3
	TOTAL	**4,494**
Ethiopia	EIAR-Melkassa	No accessions reported but details on in vitro
Guadeloupe	CIRAD	No accessions reported but details on in vitro
India	NRCB	No accessions reported but details on in vitro
Indonesia	ITFRI	No accessions reported but details on in vitro

Table D.8. *Origin of the accessions in the* ex situ *collections*

Country	Institute	From own country	From farmers	From Breeding Programmes	Introduced	No. of Acc.
Global	ITC	0%	0%	0%	100%	1404
Nigeria	IITA-NI	0%	5%	0%	27%	551
Uganda	IITA-UG	0%	0%	15%	60%	217
Australia	DAFF-Mar	0%	5%	2%	93%	417
Australia	DAFF-SJ	1%	10%	3%	87%	200
Brazil	EMBRAPA	5%	5%	0%	85%	650
Cameroon	CARBAP	0%	12%	11%	67%	650
China	IFTR-GDDAS	4%	6%	4%	36%	594
China	TBRI	0%	1%	2%	46%	446
China	TSFR Lab	7%	21%	12%	5%	220
Colombia	CORPOICA	0%	26%	0%	21%	352
Colombia	FEDEPLATANO	-	13%	71%	12%	161
Congo DRC	FSK	0%	73%	2%	5%	128
Congo DRC	INERA	4%	74%	1%	21%	68
Cook islands	MoA	3%	43%	0%	54%	35
Costa Rica	CORBANA	2%	-	-	72%	135
Côte d'Ivoire	CNRA	0%	26%	0%	74%	58
Cuba	INIVIT	14%	7%	10%	33%	544
Ethiopia	EIAR-Jimma	-	30%	-	67%	91
Ethiopia	EIAR-Melkassa	-	93%	7%	-	107
Fiji	Sigatoka	0%	30%	0%	35%	20
French Polynesia	SDR-FPNC	1%	91%	-	-	193
French Polynesia	SDR-PRFC	-	46%	-	54%	48
Guadeloupe	CIRAD	-	3%	-	97%	391
India	KAU	2%	63%	16%	13%	320
India	NBPGR	5%	49%	2%	43%	415
India	NRCB	11%	88%	-	26%	313
Indonesia	ITFRI	3%	42%	3%	16%	262
Indonesia	IIS-RCB	10%	27%	17%	2%	230
Indonesia	IIS-PBG	9%	88%	2%	2%	129
Kenya	KARI-KISII	-	94%	-	6%	166
Kenya	KARI-Thika	-	45%	-	41%	73
Malawi	BARS	1%	99%	-	-	70
Malaysia	MARDI	27%	4%	-	2%	480
Mauritius	AREU	0%	16%	1%	58%	76
Mexico	CICY	-	19%	-	100%	162
Mexico	INIFAP	0%	62%	22%	16%	37
Papua New Guinea	NARI	0%	87%	0%	4%	241
Philippines	BPI	11%	16%	0%	42%	734
Philippines	UPLB	55%	37%	-	-	200
Puerto Rico	USDA	-	-	-	53%	320
Rwanda	ISAR	91%	0%	7%	-	117
Samoa	MAF	-	22%	-	43%	46
South Africa	ARC-ITSC	0%	37%	0%	100%	123
Sri Lanka	HORDI	5%	65%	-	-	20
Sudan	ARC	99%	1%	-	-	357

Country	Institute	From own country	From farmers	From Breeding Programmes	Introduced	No. of Acc.
Tanzania	ARI-Maruku	0%	42%	-	-	260
Togo	ITRA	-	100%	-	38%	13
Uganda	NARO	-	51%	1%	48%	452
USA	Waimea	33%	-	-	38%	58
Vanuatu	VARTC	-	13%	-	19%	154
Vietnam	FAVRI	36%	12%	-	32%	152

Table D.9. *Part of the collections that make the collection important or unique and descriptions*

Country	Collection	Description
Global	ITC	The collection covers the widest range of cultivated species from a wide range of geographic areas in particular plantains originating from West and Central Africa, Highland bananas from East Africa, cultivated and wild forms collected in PNG. The collection also holds representatives of a wide range of wild species.
Australia	DAFF-SJ	Cavendish; niche cultivars; Fe'i Breeding program hybrids
Burundi	IRAZ	Many accessions have been collected in the Countries of great lakes (BURUNDI, R.D.Congo, Rwanda) and highlands cultivars (AAA-EA/Mutika –Lujugira) are concerning in priority
Cameroon	CARBAP	Plantains. We have by far the most representative plantain collection probably worldwide
China	IFTR-GDDAS	The wild or improved diploid accessions that have traits of resistance to diseases, and abiotic stress.
China	TBRI	1. Special flavour, 2. Small and cute fruit type, 3. Strong and short pseudostem, 4. early florescence, 5. Fusarium wilt resistance, 6. high production, 7. stress resistance (Ex: stiff wind resistance)
China	TSFR Lab	ITC1282 We have used it in research activities very frequently.
Colombia	CORPOICA	Our recent germplasm collecting mission of diploids, known as bocadillo, bananito o babby banana, with 26 introductions.
Colombia	FEDEPLATANO	The Gros Michel cultivars
Congo DRC	FSK	Plantain part, because, we are in the plantain diversity zone (about 85 plantain cultivars
Congo DRC	INERA	Collection importante de banane plantain ; car deuxième aliment de base après le manioc
Cook islands	MoA	All the known and recorded plantains for the Cook Islands including the Maoli, Popoulu, and Horn plantain are in the collection except for 1 Popoulu cv. 'Akamou'. There are very few plants remaining of this cultivar and a farmer has agreed to provide the Ministry of Agriculture with a single shoot before the end of the year 2012. There are three other plantains in the collection which are introduced from other Pacific Island countries. Most of the plantains (Maoli and Popoulu) in the country has disappeared as they are either not commonly utilized by the present population (especially the young); and they are highly susceptible to banana weevil borer damage.
Côte d'Ivoire	CNRA	The importance of the collection is it richness in plantain accessions. The wild *Musa* taxa (*Musa acuminata ssp. burmannicoides (Calcutta 4)*) are important in breeding program for diseases resistance.
Ethiopia	EIAR-Jimma	Presence of Cooking bananas and plantains made it unique as we are not familiar with them in our food habit
Ethiopia	EIAR-Melkassa	In Ethiopia, bananas have been widely neglected by research and development programs. A large pool of banana germplasm is found in farmers' field and forest areas in Ethiopia. Nevertheless, this germplasm is being lost because of replacement of the crop by cereals, drought and human interference such as deforestation. Therefore, the collection of banana is vital to fulfil this gap.
Fiji	Sigatoka	PHIA bananas. Black leaf resistant cultivars
Fiji	SPC	There are 110 accessions in the Pacific banana collection. Unique because they are bananas from the Pacific and is consisted of different *Musa* types - Fe'i, Iholena and Maoli-Popoulu bananas uniquely originated from the Pacific. Under the Bioversity International/Trust global strategy conservation for *Musa*, SPC is coordinating a regional banana project for a field establishment and evaluation in French Polynesia and DNA fingerprinted by New Caledonia.
French Polynesia	SDR-FPNC	Maoli/Popoulu/ Iholena / Fei types
French Polynesia	SDR-PRFC	Maoli/Popoulu/ Iholena / Fei types
Guadeloupe	CIRAD	The collection encompasses most of the genetic diversity of *Musa*, Eumusa section. It includes a large range of diploid cultivars (>130), support of CIRAD's breeding programme.
India	KAU	Second best field collection in the country. Indigenous plantain germplasm. Plantains, dessert bananas. A false horn plantain (Big Ebanga)introduction popularized for cultivation,Yangambi km5, a dessert banana introduction popularized due to satisfactory yield and quality and high resistance to leaf spot, Fusarium wilt, weevil and corm-borers, suited for ratooning, shade tolerance. Popoulu, a unique introduction being popularized. Similar to plantain in pulp characters, similar uses. Exotic introductions-SH3640,FHIA-03,FHIA-21,TMB-5295-1 high productivity, tolerance to leaf spot.
India	NRCB	19 unique BB types, Edible diploids and the rich ABB variability in our collection make our collection very unique.

Country	Collection	Description
Indonesia	IIS-RCB	Wild species Wild collection of *Musa acuminata* is used for parents in pre-breeding program
Kenya	KARI-KISII	GT – this is an AAAB genome dessert banana obtained from a farmer who got a seed from AAB Sukari Ndiizi fruit and planted the seed. The plant from the seed was later found to be tetraploid AAAB closely related to Sukari Ndiizi. The accession was then named Geraldine Tucker (GT) after the farmer. The collection also has several AAB accessions including the Silk, Mysore, Prata and Sukari Ndiizi AAB groups of dessert bananas. Other accessions in the collection are East African cooking, south east Asia cooking, beer, plantain and AAA dessert bananas.
Kenya	KARI-Thika	More attention is given to indigenous cultivar which is endangered mainly due to their lack of commercial appeal. We have diploid which perhaps can be used in Breeding purposes. A cultivar locally known as Muraru cannot be found anywhere else
Malawi	BARS	The ones with red / purple skinned fruits
Malaysia	MARDI	Mostly the collections are from Malaysia and each state will be represented by at least 3 local cultivars with different traits either for fresh or process consumption
Mauritius	AREU	1. Cavendish banana (Petite Naine, Grande Naine, Cav901, Williams, Ollier. 2. “Silk” and “Sucrier”. 1. is the main species grown on a commercial scale. Its conservation assists in regular comparative studies and serves as a source of mother plants for mass multiplication. 2. These accessions are regularly introduced and evaluated because there is an increasing demand for that type of dessert banana which is very popular in Mauritius.
Mexico	CICY	In Mexico approximately 90 % of our collection can only be found at this germplasm bank. The first (babies) and last (elders) food a Mexican eats is a banana or plátano macho, so we believe that with more work of the scientific society, we can help developing programs to reduce the 7.2 million people affected by food poverty living in urban areas (cities with 15,000 or more inhabitants), and 12.2 million residing in rural areas of the country. In rural areas smallholders make use of bananas and plantains as part of their family diet and extra income using the banana leaves. That’s why new germplasm with better nutritional qualities and better biotic an abiotic stress resistance are so important for us, just as the Zea mays from Mexico to the world has been since...until now.
Nigeria	IITA-NI	AAB – plantain group. Contain west-African plantain diversity, and IITA improved diploids and tetraploids with black Sigatoka resistance and good combining ability ; high yielding BLS-resistant triploids of potential value as future cultivars
Papua New Guinea	NARI	Some accessions with yellow/orange flesh that have been analysed and showed high levels of carotenoids. Few accessions show tolerance to dry, water logging and salinity conditions.
Philippines	UPLB	Wild *Musa balbisiana* collections since they have been there in the field genebank for more than a decade but they were still uninfected with BBTV. Potential source of resistance gene for future breeding works. However, some 90% of the accession have been infected with other viruses like BBrMV and CMV. Other cooking types are also important because of their potential for processing industry.
Puerto Rico	USDA	1. Quite a few ‘True Plantains’ which are very important locally. 2. Have introduced new Sigatoka hybrids that are becoming more important. 3. Ornamentals are becoming more important. 4. Short cycle germplasm important for Southern U.S.
Rwanda	ISAR	AAA-EA – they are unique to the region of East Africa and some are possibly unique to Rwanda.
South Africa	ARC-ITSC	A large number of Cavendish selections with improved horticultural traits have been collected from producers
Sri Lanka	HORDI	Special fruit qualities and different types with some variations.
Tanzania	ARI-Maruku	It contains many of the AAA, diploids and locally grown tetraploid found in Tanzania grown in situ
Togo	ITRA	There are at less two accessions which are losing because famers don’t make interest. The raison is the accessions reach little trade value which disadvantage famers.
Uganda	IITA-UG	The East African highland bananas which are endemic to East African highlands. Wild diploids especially M. acuminata ssp. burmannicoides (Calcutta 4) and M. acuminata ssp. Malaccensis. The wild diploids are a source of resistance to many pests and diseases that affect East African highland bananas which are the primary target for improvement in the region.
Uganda	NARO	The East African highland bananas (AAA-EA). They are closely related although there are few morphological differences among the different clones.
Vanuatu	VARTC	Local cultivars of Melanesia

ANNEX E.
MGIS DATA SHARING AGREEMENT – VERSION 6 AUGUST 2012

THIS AGREEMENT is made **BETWEEN**:

1. **BIOVERSITY INTERNATIONAL**, at Parc Scientifique Agropolis II, 1990 Bd de la Lironde, 34397 Montpellier cedex 5, France ("**Bioversity**"); And
2. **[INSTITUTE]**, with address at [ADDRESS] (the "**Data Provider**").

BACKGROUND

- Bioversity undertakes, encourages and supports research aimed at enhancing the sustainable use and conservation of agricultural biodiversity to the world's most vulnerable communities through better nutrition, sustainable farming practices and conservation and use
- Bioversity is responsible for the development and management of the *Musa* Germplasm Information System (also referred to simply as "MGIS"). The purpose of MGIS is to collect and share accession level information, from *Musa* spp. collections worldwide, such as passport data, characterization and pre-evaluation data as well as photos
- This agreement concerns the collaboration between Bioversity and the Data Provider for exchange of Data from the 'Data Provider' to Bioversity during the period [Month] [Year] to [Month] [Year] (and any Data that may be provided after the date of this agreement) and its publication on the MGIS web site
- MGIS is a publicly available online database that provides information about *Musa* spp. germplasm accessions at specific genebanks to facilitate and improve their use. MGIS also has functionality to build customized queries http://www.crop-diversity.org/banana
- Targeted end users of MGIS include genebank managers, plant breeders, taxonomists, policy makers, educators, students, as well as the broader scientific community and addition to wide array of general users
- Any germplasm data supplied by the Data Provider to MGIS will be made available publicly
- MGIS will serve as a platform for publication of data provided by the Data Provider. MGIS will also offer tools for facilitating upload and data quality control. MGIS is managed in accordance with the principle that it only contains data that is publicly available.

1. DEFINITIONS

In this agreement the following words and expressions have the following meanings:

Data
Photos and passport, characterization, evaluation information concerning *Musa* spp. and any associated information including environmental information that can be derived for accessions that include collection site geo-references;

ITC
International transit Centre;

Metadata
Information concerning when, where, how and by whom Data were collected to facilitate the use and to track for improvement of accuracy of data;

MGIS
The *Musa* Germplasm Information System through which Bioversity makes available to end users the *Musa* spp. Data released and provided by the Data Provider under the terms of this Agreement.

2. BIOVERSITY'S OBLIGATIONS

Bioversity agrees to:

1. include in MGIS the Data already provided by the Data Provider up December 31, 2012
2. provide software tools, together with appropriate support, to enable the Data Provider to subsequently upload and manage their own data on MGIS after December 31, 2012
3. provide public access to MGIS via a website, and maintain this website and its user interfaces
4. manage the legal basis for access to MGIS and use of Data (disclaimers, copyright notifications, 'MGIS Portal Terms and Conditions of Use') and prominently display on the MGIS website the terms of use of the Data
5. Not alter, modify, or otherwise change the Data, except for resizing of photos. If Bioversity believes that the Data needs to be updated or/and corrected to meet set quality standards, it will inform the Data Provider so they can make the required correction(s). Bioversity may only add environmental data relating to the geo-references included in the passport information of the Data released and Metadata
6. not express any opinion on Data when making it publicly available on MGIS
7. acknowledge the source of the Data
8. require any users of data, through a notice on the 'Terms of Use' of MGIS, to give appropriate acknowledgement of the source of the Data
9. effect and maintain at its own expense, such insurance as a prudent and responsible entity would effect in relation to the MGIS and Bioversity's business
10. Release and indemnify the Data Provider from and against any loss suffered or incurred by the Data Provider as a result of a breach of this agreement by Bioversity or gross negligence.

3. DATA PROVIDER'S OBLIGATIONS

The Data Provider agrees in relation to any Data provided by the Data Provider after the date of this agreement to:

1. provide the Data to Bioversity in a format agreed by both Bioversity and the Data Provider, for Bioversity to upload to MGIS
2. upload and manage subsequent Data provided on MGIS, using utilities provided by Bioversity
3. provide MGIS with current contact details, including an email address, where requests created through MGIS, for material maintained by the Data Provider, may be forwarded
4. obtain all necessary permissions and licences from third parties, including in relation to copyright and database rights, to allow the Data to be made publicly available on MGIS

5. provide Bioversity only non-confidential data that is not subject to any restrictions for incorporation into MGIS and be responsible for any liability incurred if the Data has been provided to Bioversity in breach of such confidentiality obligations, with or without the Data Provider's knowledge
6. indemnify Bioversity against legal actions that may be brought by third parties with respect to the Data provided to Bioversity by the Data Provider for inclusion in MGIS, except to the extent that any legal action is caused or contributed to by an act or omission of Bioversity.

4. INTELLECTUAL PROPERTY

Bioversity agrees that it shall not claim any copyright or other intellectual property rights or other proprietary interest in any Data provided by the Data Provider. Bioversity agrees that it shall pass on the same restriction against claiming intellectual property or other proprietary interests over Data in the form of a 'Terms of Use' agreement to be posted on MGIS.

5. WARRANTIES

1. The Data Provider warrants that it has obtained all necessary licenses and authorizations to consent the transfer of such Data to MGIS and will assist in resolving any disputes which might arise from these issues. The assurance will be provided in writing, to Bioversity, if requested
2. The Data Provider and Bioversity will not provide any warranties for Data content, accuracy or the use of the Data and disclaim all responsibility and liability in this regard.

6. MISCELLANEOUS

1. Bioversity will make the passport, characterization and evaluation Data available to other information systems and/or portals, including GENESYS, the Global Information System on Plant Genetic Resources for Food and Agriculture as anticipated in Article 17 of the International Treaty on PGRFA (when it is created), the Global Biodiversity Information Facility, and ensure that the proper acknowledgements are given to the provider (see 2.1.7 and 2.1.8)
2. At any time the Data Provider has the right to request Bioversity to remove, within one (1) month of the Data Provider's request, Data that it previously supplied for the purpose of publication on MGIS. Such requests shall be made to Bioversity in writing
3. The Data Provider may make available, use or publish the Data elsewhere and for any purpose, without limitation.

7. TERM AND TERMINATION

1. This agreement shall be in full execution from the date the last party has signed the agreement and until terminated in accordance with clause 7.2
2. Either party, Bioversity or Data Provider, can terminate this agreement by giving a two months' written notice to the other party.

8. SETTLEMENT OF DISPUTES

1. Any dispute, controversy or claim arising out of, or relating to the Agreement, or the breach, termination or invalidity thereof arising from the interpretation or execution of this agreement ("Dispute") shall be settled amicably by negotiations between the parties
2. Any Dispute that cannot be resolved by negotiation shall be submitted by either party to international arbitration in accordance with the UNCITRAL Arbitration Rules. The place of arbitration shall be Rome, Italy, and the language to be used shall be English.

IN WITNESS WHEREOF, the Parties hereto have signed this agreement on the date below:

Signed for and on behalf of **Bioversity**:	Signed for and on behalf of institute **[INSTITUTE]**:
Name: Dr. Nicolas Roux	Name: [NAME]
Position: Genetic Resources Conservation and Use Theme Leader, Commodity Systems and Genetic Resources Programme	Position : [TITLE]
Bioversity International	
Signature:	Signature:
Date:	Date:

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Acronyms

A

ABS Access and Benefit-Sharing- part of the MLS

AgTrials The Global Agricultural Trial Repository

AOCC Afrian Orphan Crops Consortium

ARC Agricultural Research Corporation, Sudan

ARC-ITSC Institute for Tropical and Sub tropical Crops of the Agricultural Research Council

AREU Agricultural Research and Extension Unit, Mauritius

ARI Agricultural Research Institute, Tanzania

B

BAPNET Banana Asia-Pacific Network

BARS Department of Agricultural Research and Technical Services

BARI Bangladesh Agricultural Research Institute

BARNESA Banana Research Network for Eastern and Southern Africa

BBTD Banana Bunchy Top Disease

BBrMV Banana Bract Mosaic Virus

BBTV Banana Bunchy Top Virus

BGCI Botanic Gardens Conservation International

Bioversity Bioversity International (formerly INIBAP, IPGRI and IBPGR), Italy

BLS(D) Black Leaf Streak (disease) also known as Black Sigatoka

BPI Bureau of Plant Industry, Philippines

BRIS Banana Research Information System

BSIMV BSV-Imové virus

BSGFV BSV-Goldfinger virus

BSOLV BSV-Obino l'Ewai viru

BSV Banana streak virus

C

CARBAP Centre Africain de Recherche sur Bananiers et Plantains, Cameroun

CARDI Cambodian Agricultural Research and Development Institute, Cambodia

CBD Convention on Biological Diversity

CCAFS CGIAR Research Program on Climate Change, Agriculture and Food Security

CENAREST Centre National de la Recherche Scientifique

CEPACT Centre for Pacific Crops and Trees

CGIAR Consultative Group on International Agricultural Research

CGKB Crop Genebank Knowledge Base of the CGIAR

CIAT International Centre for Tropical Agriculture

CIFOR Center for International Forestry Research

CIRAD Centre de coopération internationale en recherche agronomique pour le développement, France

CMV Cucumber mosaic virus

CNRA Centre National de Recherche Agronomique, France

CORAF Conseil Ouest et Centre Africaine pour la Recherche et le Developpement Agricoles

CORBANA Corporación bananera nacional, Costa Rica

CORPOICA Corporacíon Colombiana de Investigacíon Agropecuaria, Colombia

CRB-PT Centre de Ressources Biologiques Plantes Tropicales CIRAD-INRA

CRP CGIAR Research Programme

CRP-RTB CRP on Roots, Tubers and Bananas for Food Security and Income

CSIR-CRI Council for Scientific and Industrial Research - Crops Research Institute

CTG MusaNet Conservation Thematic Group

CWR Crop wild relatives

D

DAFF Maroochy Department of Agriculture , Forestry and Fisheries, Queensland Government, Maroochy, Australia

DAFF South Johnstone Department of Agriculture , Forestry and Fisheries, Queensland Government, South Johnstone, Australia

DARTS Diversity Arrays Technology

dCAPs Derived Cleaved Amplified Polymorphic Sequence (molecular marker)

DGD Belgian Direction générale Coopération au développement et Aide humanitaire

DSA Data Sharing Agreement

DTG MusaNet Diversity Thematic Group

E

EAHB East-African Highland AAA bananas

eBSV Episomal banana streak virus

EIAR-Jimma Ethiopian Agricultural Research Institute, Jimma Research Center

EIAR-Melkassa Ethiopia Institute of Agricultural Research, Melkassa Agricultural Research Center

ELISA Enzyme-linked immunosorbent assay

EMBRAPA Empresa Brasileira de Pesquisa Agropecuária (Brazilian Enterprise for Agricultural Research)

ETG MusaNett Evaluation Thematic Group

F

FAO Food and Agriculture Organization of the United Nations

FAVRI Fruit and Vegetable Research Institute, Vietnam

FC CGIAR Fund Council

FHIA Fundación Hondureña de Investigación Agrícola (Honduran Agricultural Research Foundation)

Foc Fusarium oxysporum var. cubense

FV Field Verification

G

GBIF Global Biodiversity Information Facility

GBS Genotyping by sequencing

GCP Generation Challenge Programme

GDAAS Guangdong Academy of Agricultural Sciences

GDCT Global Crop Diversity Trust

GENESYS the Global Information System for Plant Genetic Resources for Food and Agriculture

GMGC Global Musa Genomics Consortium

GPA Global Plan of Action

GRIN Germplasm Resources Information Network, USA

GRIN-GLOBAL Global version of the Germplasm Resource Information Network system

GSPC Global Strategy for Plant Conservation

GTG MusaNet Genomics Thematic Group

GWAS Genome-wide association study

H

HORDI Horticultural Crops Research and Development Institute

I

IAEA International Atomic Energy Authority, Austria

ICHORD the Indonesian Centre for Horticulture Research and Development

ICRA Institut Centrafricain de Recherche Agronomique

ICTA Imperial College of Tropical Agriculture

IEB Institute of Experimental Botany

IFTR/GDAAS Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences

IIHR Indian Institute of Horticultural Research

IIS-PBG Purwodadi Botanic Garden – Indonesian Institute of Sciences

IIS-RCB Indonesian Institute of Sciences, Research Center for Biology

IITA International Institute of Tropical Agriculture, Nigeria

IMTP International Musa Testing Programme

INERA-RDC Institut National pour l'Etude et la Recherche Agronomique - Yangambi

INIBAP International Institute of Banana and Plantain

INIFAP Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

INIVIT Instituto de Investigaciones de Viandas Tropicales

INRA Institut National de Recherche Agricole

IPB Institute of Plant Breeding, Philippines

IPGRI International Plant Genetic Resources Institute (now Bioversity International)

IPM Integrated pest management

IPPC International Plant Protection Convention

IRAZ Institut de recherches agronomiques et zootechniques de la CEPGL, Burundi

IRD Institute de Recherche de Développement

ISAR Institut des Sciences Agronomiques du Rwanda

ISH in situ hybridisation (FISH and GISH)

ITC Bioversity International Transit Centre

ITFRI Indonesian Tropical Fruit Research Institute, Indonesia

ITG MusaNet Information Thematic Group

ITPGRFA International Treaty for Plant Genetic Resources for Food and Agriculture

ITRA Institut Togolais de la Recherche Agronomique

ITSC Institute for Tropical and Sub tropical Crops

IUCN International Union for the Conservation of Nature

J

JCVI J. Craig Venture Institute

K

KALRO-KISII Kenya Agricultural and Livestock Research Organisation

KARI Kenya Agricultura Research Institute

KAU Kerala Agricultural University

KULeuven Katholieke Universiteit Leuven, Belgium

L

LTS Long-term storage (cryopreservation at ITC)

M

MAF Ministry of Agriculture and Fisheries

MARDI Malaysian Agricultural Research and Development Institute

MAS Marker assisted selection

MCPD MultiCrop Passport Data

MGBMS Musa Gene Bank Management System of the ITC

MGC Musa Genotyping Centre

MGIS Musa Germplasm Information System

MLS Multilateral system (of access and bernefit sharing)

MoA Memorandum of agreement/ Ministry of Agriculture

MOOS Musa On-line Ordering System

MTS Medium -term storage (in vitro at ITC)

MUSALAC Plantain and Banana Research and Development Network for Latin America and the Caribbean

MusaLit Musa Literature database of ProMusa

MusaNet Global Musa Genetic Resources Network

MusaPedia Knowledge Base of ProMusa

N

NARI National Agricultural Research Institute

NARO National Agricultural Research Organisation, Uganda

NARS National Agricultural Research Station

NBPGR National Bureau of Plant Genetic Resources, India

NGO Non-governmental organisation

NGS Next generation sequencing

NRCB National Research Centre for Banana, India

NRMDC National Repositories Multiplication and Distribution Centres

O

OLGA Outil Locale pour la Gestion des Accessions

OT Off Type

P

PAPGREN Pacific Plant Genetic Resources Network

PCR Polymerase chain reaction

PDCI Passport Data Complexion Index

PGRFA Plant genetic resources for food and agriculture

PKW Pisang Klutuk Wulung (Musa cultivar)

PPRI Plant Protection Research Institute, South Africa

PRFC Pacific Regional Field Collection

ProMusa Knowledge-sharing platform on bananas managed by Bioversity International

Q

QMS Quality Management Systems

R

RADseq Restriction-site Associated DNA sequencing

RMDC Regional Multiplication and Distribution Centres

RTB CRP on Roots, Tubers and Bananas

S

SDR-MAP Service du développement rural, French Polynesia, Tahiti

SMTA Standard Material Transfer Agreement

SNPs Single nucleotide polymorphisms

SRS Sigatoka Research Station - Ministry of Agriculture

SPC Secretariat of the Pacific Community, Pacific Islands

SSR Simple Sequence Repeats

SupAgro Institut national d'études supérieures agronomiques

T

TAG Taxonomic Advisory Group

TBRI Taiwan banana research institute

TSFR Lab South China Agricultural University

TGSMG Technical Guidelines for the Safe Movement of Germplasm

TRC Taxonomic Reference Collection

Trust The Global Crop Diversity Trust, Germany

TSFR Tropical and Subtropical Fruit Research Lab. China

TT True to Type

TTC tetrazolium chloride

U

ULg University of Liege, Belgium

UPLB University of the Philippines Los Baños, Philippines

UQ University of Queensland

USDA United States Department of Agriculture, USA

USDA-TARS United States Department of Agriculture, Tropical Agriculture Research Station, Puerto Rico, USA

V

VARTC Vanuatu Agricultural Research and Technical Centre

VIC Virus Indexing centre

W

Waimea Waimea Valley Arboretum and Botanical Garden

GLOSSARY OF TERMS

The definitions are focused on *Musa* wherever possible or relevant.

Allele
One of the (usually two) alternative forms of a gene, found at the same corresponding location on a corresponding chromosome.

Allopolyploidy
Polyploidy produced by the hybridization of two species[1]. Many banana cultivars are allotriploid hybrids of *Musa acuminata x M. balbisiana*.

Aneuploid
An individual with additions or deletions of whole chromosomes from the expected, balanced number of chromosomes.

Backcross
The cross of an individual with one of its parents or a taxon with the same genotype as a parent.

Biodiversity
The total variability within and among of all living organisms and their habitat.

Centre of diversity
Geographic region with an exceptional variability of a crop. The primary centre of Musa diversity concerns the wild species and the domesticates generated in or nearby it. Secondary centres of diversity consist of cultivars generated beyond the primary centre, mostly by somatic mutations[2].

Character
An attribute of an organism resulting from the expression of a gene or a set of genes.

Characterization
The description of a taxon by morphological and molecular markers, in order to distinguish it from other taxa.

Chimera
A plant which contains two dissimilar tissues (or cell lines) for the same character.

Cigar leaf stage
The early stage of leaf emergence in which the lamina is rolled up into a cylindrical shape. This stage ends when the lamina opens.

Clade
A group of organisms believed to comprise all the evolutionary descendants of a common ancestor.

Clone
A group of cells, tissues or plants descended by mitosis from a single ancestor (cell, tissue or plant). Banana cultivars are frequently called ‘clones’.

Clone set
A set of cultivars within a subgroup that share one or more characters which are different in other members of the subgroup. The latter can form one or more other clone sets. An alternative name, ‘cluster’, is also applied but is commonly used for molecular marker results (e.g. in cladograms).

Corm
Alternative horticultural/agronomic name for the rhizome.

1 Tamarin

2 Authors’ attempt to meet the common use of only two divisions (primary/secondary)

Crop Ontology
The Crop Ontology (CO) compiles plant traits and their variables along with definitions, methods and scales of measurement, that are validated by crop scientists and are useful for data annotations and producing field-books in breeding, phenotyping, agronomy and genebanks. Traits are grouped in the following classes: abiotic stress, agronomic traits, biotic stress, morphological traits, phenological traits, physiological traits, quality traits.
This discipline was primarily developed to serve the crop breeders' field book and is progressively being extended to other types of traits.
A similar term- 'Plant Ontology' can be defined as the set of terms for organs and tissues that are common to many crops. This is a species agnostic ontology about the anatomy and the development stages of plants and is maintained by Oregon State University.

Cultivar
An assemblage of edible banana plants that has a same combination of all characters, is distinct, uniform, and stable in these characters, and retains the combination via clonal propagation. See difference with 'variety', 'landrace', and 'subgroup'.

Descriptor
A standardized list of terms used to document the characters, the environment, the management and other parameters linked to an accession.

Diversity Arrays Technology (DArT)
A high throughput genotyping technology enabling the detection of presence versus absence of genome fragments.

Epigenetic(s)
Refers to changes in the regulation of gene activity and expression that are not dependent on gene sequence[3].

Flow cytometry
A technique based on fluorescence allowing the measurement of the nuclear DNA content and allowing ploidy level determination.

Form
Subdivision of a variety. In the genus *Musa*, where even the varieties remain to be duly identified, the presumed forms have as yet not been explored. Also used for morphology, as in form and function.

Gamete
A reproductive cell resulting from meiosis that unites with another cell to form a new individual through fertilization.

Gene
The functional unit of heredity. A gene is a section of DNA that codes for a specific biochemical function in a living organism.

Genepool
All of the alleles available among the reproductive members of a population from which gametes can be drawn.

Genetic diversity
The variation present in a group of individuals, populations or species that is due to genetic or epigenetic differences [as opposed to the expression of the same (epi-) genetic background in different environments.

Genetic erosion
Loss of genetic diversity between and within populations of the same species or the loss of entire species (e.g. wild relatives) over time, or reduction of the genetic base of a species due to human intervention, environmental change etc.

3 http://www.roadmapepigenomics.org/overview

Genetic marker
A fragment of DNA that is associated with a certain location within the genome. The polymorphism in genetic markers points to genetic diversity (see also 'Polymorphism').

Genetic resources
Genetic materials of plants, animals and other organisms which is of current or potential value as a resource from a genetic perspective.

Genome
A set of chromosomes corresponding to the haploid set of a species.

Genotype
The entire genetic constitution of an individual (banana) plant- the genetic background of the phenotype.

Genotyping
The process of determining the genetic constitution – the genotype – of an individual by examining their DNA sequence.

Genotyping by sequencing (GBS)
An advanced technology (based on Next Generation Sequencing, NGS) that allows to decipher the genotype at the nucleotide level and to discover Single Nucleotide Polymorphism (SNP) between individuals.

Genus (Genera in plural)
A category of biological classification ranking between 'family' and 'species', comprising structurally or phylogenetically related species.

Germplasm
See 'Genetic resources' However, in relation with Conservation, it may be defined as 'The living genetic resources maintained for the purpose of animal and plant breeding, preservation, and other research uses'[4].

Group
(Alternatively: genome-group). The highest rank in the classification of edible bananas, typified by a particular combination of the constitutive genomes of *Musa acuminata* (A) and *Musa balbisiana* (B). Examples: AA, AB, AAB, ABB. In rare cases, the Group contains also a genome of another species such as *M. schizocarpa* (S) and *M. textilis* (T).

Habitat
The specific place or area where a particular banana population (usually a variety) is nearly uniform with almost the same genotype In evolutionary terms: the place or area where that population originated.

Haploid
The state of having one copy of each chromosome per nucleus.

Heterosis
A marked vigour often shown by crossbred plants.

High density array
Increasing the number of genes to analyse simultaneously through reducing DNA spot size and bringing them closer together. (See also micro array).

Holotype
A specimen commonly recognized as representing a wild taxon (generally a species or subspecies).

Homology
The state of two chromosomes having the same complete sequence of genes[5].

4 Wiki adapted

5 Tamarin adapted

Homonym
A taxonomic designation rejected as invalid because the identical term has been used to designate another group of the same rank[6].

Hybrid
The offspring of two plants or animals of different species or varieties.

Identification
The classification of a duly characterized taxon.

Landrace
In general: a selected population in out-cross plants developed by local farmers. For edible bananas, the term is erroneously used in some regions instead of 'cultivar' (which is a clone).

Leaf
An expanded outgrowth of the stem and usually containing a bud at its base (where the leaf arises from the stem). A banana leaf consists of a 'leaf sheath, a petiole, and the 'leaf blade'.

Leaf sheath
The basal part of a banana leaf. The enrolled sheaths form together the pseudostem of a banana plant.

Leaf blade
The laterally expanded upper part of a banana leaf (also called 'lamina; the term 'limb' should be rejected).

Marker-assisted selection
Selection of genetic material based on the possibility of detecting the presence of a gene or agronomic trait of interest by the search of a biochemical or molecular marker that is closely linked to it.

Meiosis
The nuclear process that results in the production of gametes.

Microarray
Collection of microscopic DNA spots attached to a solid surface used to analyse a large number of gene simultaneously (see also High density array).

Modern cultivar
An end-product of genetic improvement of banana, accepted by consumers and clonally propagated by farmers.

Modern variety
Non-correct term for bananas. See 'Modern cultivar'.

Molecular marker
Has a broader meaning than 'Genetic marker' but frequently used as alternative term.

Morphology
The science of form and structure of plants (and animals).

Morpho-taxonomic description
Description of the plant made by observing the morphological characters. The description should always be related to the environment in which the plant is growing because some of the characters are expressed differently according to the environment.

Morphotype
The set of visible characters that typify a taxon.

***Musa* core collection**
A collection of minimum representative *Musa* diversity.

6 Webster

Musa mini-core collection
A core collection composed of 52 accessions (DNA samples used for molecular studies[7]. (Linked to the Taxonomic reference collection- see below).

Next Generation Sequencing
Advanced high-throughput DNA sequencing technologies. See 'Genotyping by sequencing' and 'Restriction-site Associated DNA sequencing'.

Neo-allotetraploid
Genotypes obtained by joining the genomes of two close species, through hybridization and chromosome doubling. Depending on meiotic behaviour, these genotypes could be sexually reproduced.

Nomenclature
System of naming taxa.

Omics research
Aims at the collective characterization and quantification of biological molecules (mainly nucleic acids, proteins, and metabolites) that translate into the structure, function, and dynamics of an organism (e.g. proteomics, transcriptomics or metabolomics).

Pan-genome
Describes the full complement of genes in a clade (see above), which can have large variation in gene content among closely related strains. It is composed of all the genes present in at least one specimen/ strain of a given clade (e.g. species).

Parthenocarpy
The development of a fruit without seed formation

Peduncle
The exposed part of the banana stem that supports the flowers/fruits. The portion at its base without flowers can be named the 'transitional peduncle'. The female peduncle supports the fruit, the male peduncle supports male flowers that may dehisce.

Petiole
The middle part of the banana leaf which connects the sheath to the mid-rib of the leaf blade.

Phenotype
The sum of the characters of an individual as the expression of its genotype. The term covers morphological, physiological and agronomical characters. Thus the sum of Morphotype + Quantitative traits + Physiotype (non-visible characters playing a role in the physiology of a plant).

Phylogeny
The evolution of a genetically related group of organisms.

Plantain (true-)
A very large AAB subgroup, mainly in Africa where it is a basic food-crop in the rainforest region. The fruits are generally only palatable after cooking or roasting.

Ploidy
The number of basic sets (x) of chromosomes in a cell, tissue or plant. (Haploid=one, diploid =2; triploid=three; tetraploid=4 etc.)

Polymerase chain reaction (PCR)
A technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders.

7 See https://sites.google.com/a/cgxchange.org/musanet/genotyping-centre/genomic-dna

Polymorphism
A discontinuous genetic variation resulting in the occurrence of several different forms or types of individuals among the members of a single taxon. In Morphology, the term corresponds with descriptors of the members. In molecular genetics, biochemical techniques can reveal not-visible manifestations and identify differences that occur between the chromosomes, proteins, or DNA of different forms polymorphism.

Polyploid
With greater than two chromosome sets.

Population
An undefined taxon in a text. A heterogeneous assemblage of cultivars and/or varieties. Also: any not yet classified banana assemblage in a particular location.

Pseudo-gene
A gene that became inactive, usually as the result of genetic mutations causing it to lose its ability to encode proteins or to be expressed within a cell.

Pseudostem
The 'false stem' of a banana plant, i.e. the orderly assembly of many overlapping leaf sheaths, which forms a massive fibrous column, in the centre of which the true aerial stem elongates after floral induction and emerges as the peduncle with the inflorescence.

Quantitative attributes
(Also known as 'quantitative traits' (QTs) or 'continuous traits'). For example: 'amount', 'dimension', 'weight' 'degree of resistance', mostly in absolute terms. Prominent role in breeding and evaluation.

Restriction-site Associated DNA sequencing (RADseq)
Type of molecular marker based on sequencing regions close to specific restriction sites. Since the restriction sites are randomly distributed along the genome, this analysis allows exploring the whole genome of a large number of individuals by Next generation Sequencing, at reduced cost.

Reference Collection
A set of 35 accessions that represents the essential morpho-taxonomic diversity in edible bananas.

Rhizome
The massive and starchy underground stem of a banana plant. See also 'corm'.

Simple Sequence repeat (SSR) markers
Markers based on variations in the number of short repeated nucleotide sequences –microsatellite repeats-. Their high mutation rate enables the detection of recent polymorphism between closely related accessions.

Single nucleotide polymorphism (SNP)
Variation (polymorphism) within the genome of a single base pair between individuals.

Somaclonal variation
Variation observed in plants regenerated from in vitro culture and which do not conform to the original plant. Some such variants closely resemble other cultivars of a same subgroup. Sometimes erroneously used for 'somatic mutation'.

Somatic mutation
Variation in the tissue-relevant genotype expression of a cultivar, maintained via clonal propagation as a new cultivar.

Species
A group of wild bananas capable of interbreeding freely with each other but usually not with members of other species. In taxonomic classification, a subdivision of a genus.

Subgroup
(for bananas) A subdivision of a Group for a set of cultivars that are assumed to have been generated from a common ancestor by somatic mutation. The ancestor is assumed to be a hybrid between two distinct parents (proved in the case of Cavendish subgroup).

Subspecies
Subdivision of a species consisting of varieties growing in a more or less defined region.

Sucker
Ramification of the rhizome appearing at the base of the parent plant, usually consisting of small leaves with more or less reduced blade. See also 'sword sucker' and 'water sucker'. Suckers are the outgrowth of lateral buds previously formed at the leaf base.

Sword sucker
A vigorous sucker at a young stage characterized by laterally- and heavily reduced sword-shaped lamina. When in a later stage several leaves with more developed lamina ('foliage') appear, the sucker is named 'maiden sucker'.

Sympatric
Having the same, or largely overlapping, areas of geographical distribution. Opposite: 'allopatric'

Synonym (of cultivar name)
Any full name that has been applied to a particular taxon that differs from the taxon name. Numerous cultivars have almost as much such different names as there exist languages in the regions where they a popular.

Systematics
See 'Taxonomy'.

Taxon (pl. taxa)
Any definite unit in classification of plants and animals.

Taxonomic reference Collection (TRC)
Originally 33 accessions but now it is composed of 29 accessions. In both case they represent the diversity of Musa even though it is mainly cultivated type diversity with addition of very few wild types[8].

Taxonomy
The botanical discipline for the identification and classification of taxa. 'Systematics' is frequently used as alternative name.

Trait
Qualitative (see character) and quantitative attributes or features of a phenotype.

Transgenic modification
Genetic re-combinations produced by introducing genes from other genera/species via molecular biotechnology.

Variety
A lower rank subdivision of a (wild) species, with restricted habitat. See also 'subspecies' and 'form'.

Vegetative propagation
Asexual/clonal propagation by which a banana plant is produced which is identical in genotype with the parent plant.

Water sucker
A fragile sucker that underwent premature weaning from the mother plant. It forms very small leaves with relatively large blades and a ramified rooting system as response (compare with 'sword sucker'. When transplanted and maintained with special care, it can develop as a normal plant.

Wild relative
A wild taxon which is presumed to have genetically contributed to the genome of a cultivar.

8 http://www.promusa.org/Taxonomic+Reference+Collection

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ISBN: 978-92-9255-050-9

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