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The management of banana (*Musa* spp.) genetic resources at the IPGRI/INIBAP gene bank: the conservation and documentation status.

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Abstract

The international banana germplasm collection is managed by the IPGRI/INIBAP Transit Centre in Belgium since 1985. This unique collection, placed under the auspices of FAO in 1994, consists of approximately 1200 accessions of wild, cultivated and improved bananas, introduced from 44 countries in the world. Samples of these accessions are permanently maintained *in vitro* as proliferating shoot cultures under slow growth conditions at low temperature (16°C) and reduced light intensity (25µmol/m²/s). Incoming germplasm undergoes a standardized indexing process for five virus diseases in collaboration with 3 indexing centres (Australia, France and South Africa) and pathogen-free accessions are made freely available for international distribution. Throughout the last five years, over 25,000 samples of germplasm have been delivered worldwide to hundreds of institutes and individuals involved in development projects with farmers, for various research activities or to underpin specific gene bank activities such as cytological studies and virus eradication research. Currently, also a long-term base collection is being established, using a cryopreservation protocol developed at the Laboratory of Tropical Crop Improvement, KULeuven, Belgium. At present, more then one quarter of the entire collection is safely stored in liquid nitrogen (-196°C). Evidently, the effective management and use of this unique collection strongly depends on its documentation status. To facilitate data maintenance and processing relevant for the day-to-day activities of the gene bank, a tailored-made information system using bar-codes has been put in place recently. All passport data, and available characterization and evaluation data are documented in the INIBAP's *Musa* Germplasm Information System (MGIS). This decentralized system contains standardized information on banana varieties managed in 18 banana gene banks around the world and is readily accessible for users (mgis.grinfo.net or mgis.inibap.org). The data of the INIBAP Transit Centre, which are part of the MGIS database, are also available through SINGER (System-Wide Information Network on Genetic Resources), an on-line database containing information on genetic resources held by the CGIAR Centres (singer.cgiar.org).

Introduction

Banana (*Musa* spp.) is one of the most ancient fruit crops known and used by man. The genus originated in South-East Asia and was domesticated first in Papua New Guinea, probably more than 7000 years ago (Denham *et al*., 2003). Later on the crop was spread to the African continent (De Langhe, 1995) where two secondary centres of landrace diversity developed, the plantains (AAB) evolved in West- and Central-Africa and the highland bananas (AAA-EA) in East-Africa.

Today, banana is grown in every humid tropical and many sub-tropical regions and ranks fourth as the world's most valuable food crop, behind rice, wheat and maize (INIBAP, 2005). Almost 95 million metric tons (MT) of which 30 million MT plantains, are harvested annually around the world (INIBAP, 2004). The fruit crop provides a staple food source for 400 million people and reaches its greatest importance in parts of East-Africa where annual consumption is over 200kg/capita (FAOSTAT data, 2005). About 90% of the total production takes place on small-scale farms and is used for home consumption or goes to domestic markets (INIBAP, 2004). The remaining 10%, consisting of a few dessert banana types only, is produced on commercial plantations in Latin-America and the Caribbean mainly, and enters the world trade (INIBAP, 2005).

Recognizing the importance of bananas as a staple food and a vital source of income for small growers in several developing countries, the International Network for the Improvement of Banana and Plantain (INIBAP), the banana branch of the IPGRI (International Institute on Plant Genetic Resources) 'Commodities for Livelihoods' Programme started in 1985 coordinating and supporting the international effort for collecting, preserving, documenting and enhancing the use of banana genetic resources.

The INIBAP *Musa* gene bank

The international Musa germplasm collection

Since 20 years, INIBAP holds the most comprehensive collection of banana in the world. The gene bank, managed at the INIBAP Transit Centre (ITC), housed at the Laboratory of Tropical Crop Improvement at KULeuven, Belgium, counts almost 1200 accessions, introduced from 44 countries in the world (Table 1). The collection covers all categories of cultivated materials, a wide range of wild relatives, and elite germplasm produced by breeding programmes worldwide. After the establishment of the INIBAP Transit Centre (ITC) in 1985, major banana field gene banks started sending duplicate samples of their accessions to Leuven for back-up storage. In the late 80s, IPGRI/INIBAP took the responsibility of coordinating collecting missions in unexplored areas of diversity in order to broaden the exploitable genetic base. Within the framework of four missions jointly executed by IPGRI and QDPI (Queensland Department of Primary Industries) that took place in Papua New Guinea, more than 300 unique accessions were collected (Sharrock, 1990). Once a specimen has been collected, it is conserved by a national institution in the country of origin and duplicates of the samples are deposited at the IPGRI/INIBAP gene bank. In the 90s more original materials were collected in Vietnam, Indonesia, China, India, Tanzania and Oman and they are progressively included in the global *Musa* collection. The collection was also further enriched with a range of improved cultivars produced by several breeding programmes in the world (Table 1).

In 1994, the collection was placed under the auspices of FAO within the International Network of *ex situ* Collections ensuring the long term storage of holdings and unrestricted access by the world community.

Table 1. Holdings of the international *in vitro* collection of banana and their origin or donor source.

	Origin/Donor	Number of accessions	Genotypes
Collecting Missions	Papua New Guinea (1989-1990)	278	diploid wild/cultivated forms
	Vietnam (1996)	43	wild/cultivated forms
	Tanzania (2002)	21	East African highland bananas
Collections	Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Guadeloupe and France (1987-1990)	233	wild/cultivated forms
	Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica (1986-1989)	23	cultivated forms
	Fundación Hondureña de Investigación Agrícola (FHIA), Honduras (1988)	126	wild/cultivated forms
	Institut de Recherches Agronomique et Zootechnique (IRAZ), Burundi (1987)	54	East African highland bananas
	International Institute of Tropical Agriculture (IITA), Nigeria (1986-1987)	115	AAB-plantains
Breeding programmes	Centre Africain de Recherche sur Bananiers et Plantains (CARBAP), Cameroon	114	improved cultivars
	Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Guadeloupe		
	Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), Brazil		
	Fundación Hondureña de Investigación Agrícola (FHIA), Honduras		
	Instituto Nacional de Investigaciones en Vinades Tropicales (INIVIT), Cuba		
	International Atomic and Energy Agency (IAEA), Austria		
	International Institute of Tropical Agriculture (IITA), Nigeria		
	Taiwan Banana Research Institute (TBRI), Taiwan		
Others (other collections, botanical gardens, private persons,...)		168	wild/cultivated forms

Operation of the banana gene bank

New accessions are usually received at the ITC as small samples of *in vitro* cultures, classical vegetative propagation material (young suckers) or as seeds. Initially, from each new sample an aseptic shoot culture is established. A selection of the most vigorous one from which a sub-clone of seven cultures will be established, is made. A set of five cultures is sent to an INIBAP Virus Indexing Centre (VIC) at CIRAD (Centre de coopération internationale en recherche agronomique pour le développement), France, QDPI, Australia or PPRI (Plant Protection Research Institute), South Africa, where they are inspected for the presence of the five major banana viruses, CMV (cucumber mosaic cucumovirus), BBTV (banana bunchy top virus), BSV (banana streak virus), BanMMV (banana mild mosaic virus) and BBrMV (banana bract mosaic virus), following standardized indexing procedures as proposed in the FAO/IPGRI Technical Guidelines for the Safe movement of Germplasm (Diekmann & Putter, 1996). From the other two cultures, a set of 20 replicates is produced for storage under medium term storage conditions in the active collection. If the test results are negative after inspection at the VICs, the accession is cleared for distribution. In case viruses are detected, the accession is subjected to virus therapy (Helliot *et al*., 2003; 2004) and re-indexed. A virus-free line is maintained in the active collection to meet requests from clients and a subset of cultures is prepared for storage in liquid nitrogen in the base collection. A second subset of materials is regenerated under greenhouse conditions for harvesting leaf samples that are processed in a DNA/lyophilized leaf bank. The banked leaf materials serve as voucher for the germplasm stored in the active and base collection and samples are made available to users for research into gene discovery and function, marker development and detailed genotypic characterisation.

The establishment of the INIBAP Transit Centre, with the *in vitro* collection, in a non-banana growing country, linked to the INIBAP virus indexing centres activity, greatly facilitated the international exchange of banana germplasm over the past 20 years. Currently, 62% of all accessions in the active collection is certified as virus-free and has 20 multiple healthy shoot cultures that are readily accessible for distribution. Samples are made freely available so that useful diversity reaches as many researchers and eventually farmers in the developing world as possible. Standard shipments of germplasm usually contain five separate *in vitro* cultures - multiple shoot clusters or rooted plants- of each requested accession.

Since 1985, about 200 institutes in 88 countries worldwide benefited from this service. In the last five years only, over 25,000 samples of germplasm and associated information have been delivered worldwide to users involved in development projects with farmers, for various basic research activities, or to underpin specific gene bank activities such as cytological studies and virus eradication research.

Conservation of *Musa* diversity

Ex situ conservation approaches

Bananas are fast-growing herbaceous perennials arising from underground rhizomes. The wild plants that are classified as two species, *Musa acuminata* (AA) and *Musa balbisiana* (BB), set seed. These species gave origin to the common cultivated types which are characterized by female sterility, parthenocarpy and various levels of male sterility. The seedless clones which can be diploid, triploid or tetraploid, are maintained exclusively through vegetative propagation by suckers.

Since the application of seed storage in a crop such as banana is limited to the wild relatives, banana genetic resources are traditionally preserved *ex situ* in field collections. The

conservation of germplasm in these live gene banks is most suitable for characterization and breeding purposes. However, it involves tremendous risks of exposure of valuable and unique germplasm to biotic threats and environmental stresses. Moreover, such collections require regular replanting, large amounts of land space and labour input. In the early '80s, the implementation of tissue culture techniques opened new perspectives for a complementary conservation approach for vegetatively propagated crops, including banana. The application of *in vitro* storage allows conservation of germplasm in a protected environment and offers the added advantages of disease elimination, aseptic plant production, safe and easy international exchange of plant materials and lower conservation costs. Nonetheless, maintaining the material as shoot tip or meristem cultures remains labour intensive and involves the risk of losing valuable germplasm through accidental contamination of cultures and human error. Another and major impediment of storage of tissue cultures under slow growth conditions is the possibility of genetic instability due to somaclonal variation. Preservation of *in vitro* tissues at cryogenic temperatures (-196°C) whereby all metabolic processes and cell divisions are arrested, is considered as the only suitable alternative that can ensure the long term security of stored germplasm. The method also has the advantage that very limited space is required, the material is protected from contamination and very little maintenance is needed (Sharrock & Engels, 1997).

Medium and long term storage of the global Musa collection

INIBAP opted to preserve the collection at the ITC under *in vitro* medium term storage conditions with a long term back-up in liquid nitrogen. Each accession is maintained under the form of 20 proliferating shoot cultures all grown on a semi-solid Murashige and Skoog-based culture medium supplemented with 30g/l sucrose and two plant growth regulators, a cytokinin, BAP (10µM) and auxin, IAA (1µM). In order to prolong the culture life of the stored accessions, slow growth procedures are adopted. The cultures are stored at low ambient temperature (16°C) and reduced light conditions (25µM/m^2/s), requiring subculturing every 4-22 months depending on the genotypic constitution of the accession (Van den houwe *et al.*, 1995).

Of greatest importance for the effective management of the active collection is the periodic monitoring of stored accessions to determine and guarantee the quality of the stored germplasm. Cultures are checked at regular intervals for their general performance, tested for the presence of endophytic bacteria (Van den houwe & Swennen, 2000), and accessions are timely rejuvenated *in vitro* and regenerated in the field to assess their true-to-typeness (INIBAP, 2002). However, despite all preventive measures to keep the germplasm in optimal conditions, losses of plant material or of its genetic integrity inevitably occur. Therefore, the development of an efficient and reliable protocol for cryopreservation of banana is essential for long term storage of its genetic resources.

At the Laboratory of Tropical Crop Improvement research on cryostorage of banana has been conducted for many years, resulting in the development of three cryogenic techniques that allow safe and stable preservation of the entire collection (Panis & Thinh, 2001). The first two methods use highly proliferating meristem clusters that are pre-cultured on sucrose (0.4M) enriched medium during 2 weeks. Groups of pre-cultured meristem clumps are either directly frozen (simple freezing method, Panis *et al*. 2002), or subjected to a vitrification treatment i.e. freezing without lethal ice crystal formation (vitrification method, Panis & Thinh, 2001) prior to storage in liquid nitrogen. It was observed that post-cryopreservation viability rates for the simple freezing method depend on the genotype and vary from 0-4% for *Musa acuminata* and AAA-highland bananas (AAA-EA) over 19-25% for AAA, AAB dessert bananas and AAB plantains (AABpl) to 53% for ABB cooking bananas (Figure 1). Viability rates for the

vitrification method appeared to be relatively high compared to those of the first method. Post-thaw recovery rates for ABB clones remain 50% whereas increased recovery growth rates are obtained for AAB dessert bananas, AAB plantains and AAA dessert bananas (41-51%). Viability rates for the AAA-highland bananas and *Musa acuminata* accessions however remain very low (0-30%). A third method involves vitrification of tiny individual meristems. One millimeter sized shoot-tips excised from rooted *in vitro* plants are excised and frozen with the droplet vitrification technique. This results in ultra rapid freezing and thawing rates that proved to be essential for high and reproducible post-thaw regeneration rates. When this protocol was applied to 56 accessions belonging to 7 different genomic groups of *Musa* spp., an average of 52.9% post-thaw regeneration was obtained. These results were relatively genotype independent. Only wild diploid *Musa acuminata* accessions proved to be somewhat more recalcitrant towards cryopreservation though an acceptable average regeneration rate of 39% was still obtained (Panis *et al*., 2005) (Figure 1). This method, although more than 2 times more laborious compared to the previous ones, offers a good alternative for those cultivars responding unfavourably towards the freezing of highly proliferating meristem cluster.

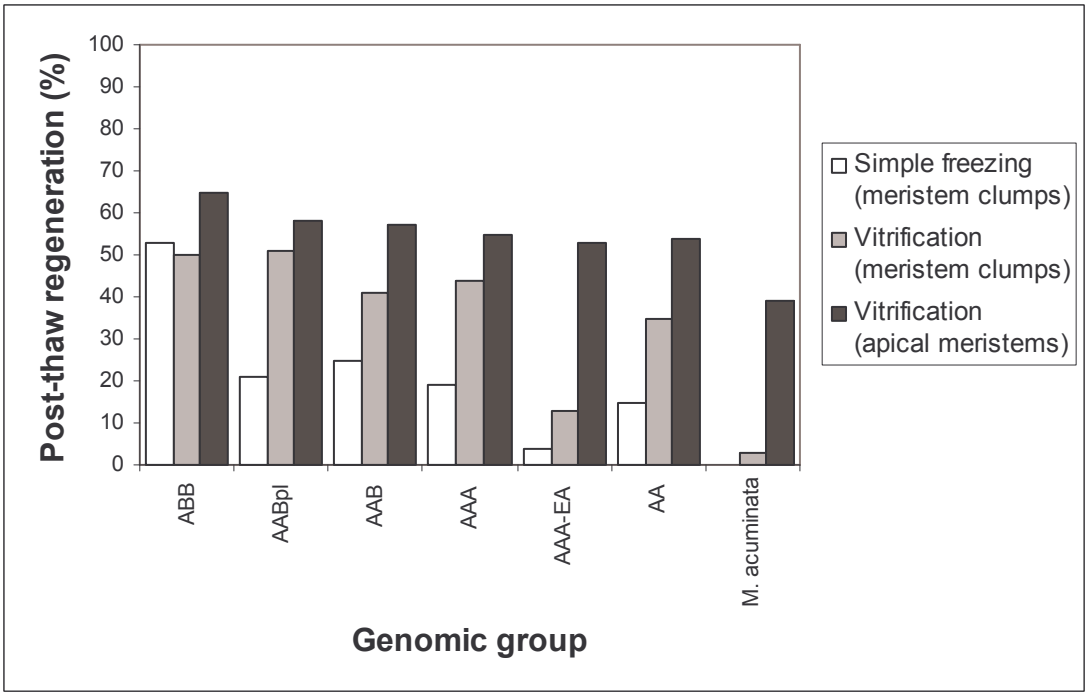

Figure 1. Average post-thaw regeneration rates of banana accessions grouped according their genomic constitution after the application of three different cryopreservation protocols.

The combination of the three techniques is now being used on a routine basis to cryopreserve the whole collection and resulted in the safe storage of 330 banana accessions belonging to the various genomic groups so far.

Documentation of banana genetic resources

Documentation status of the conserved banana germplasm

Proper documentation of germplasm accessions is essential not only to facilitate their conservation and understand the diversity but also to allow efficient utilization of a collection. It is assumed that the majority of the diversity within the genus *Musa* is represented by the

numerous field and *in vitro* collections but the uneven quality and accuracy of the related documentation is a serious drawback for its efficient use.

Many *Musa* collections, including the ITC-collection, have not been systematically documented. For the major part of preserved clones, passport data and only limited characterization and evaluation data are available. In an effort to upgrade the documentation status of the global *Musa* collection, samples of accessions are regenerated and grown in the field along-side the reference plant for taxonomic verification. Plants are systematically being described using the IPGRI morpho-taxonomical descriptors (IPGRI-INIBAP/CIRAD, 1996). Recently, the ploidy level of all accessions in the ITC collection was determined using flow cytometric (Dolezel *et al*, 1997) at the Institute of Experimental Botany in Czech Republic and a variety of genetic markers (STMS, RFLP and RAPD) are being used to conduct taxonomic studies and for gaining better insight in the organization of genetic diversity (Ford-Lloyd *et al*., 1997). The sequence-tagged microsatellite sites technique, for instance, has confirmed the group and sub-group classifications of clones of *M. acuminata*, *M. balbisiana* and *Australimusa*. This work is conducted by CIRAD-FLHOR in Guadeloupe where the technique has been applied to more than 300 accessions so far (INIBAP, 1999). Furthermore, information on disease resistance and agronomic performance of improved hybrids and natural germplasm in the collection is systematically generated through INIBAP's International *Musa* Testing Program (IMTP) (Orjeda, 2000).

Managing and sharing Musa germplasm information

Musa Gene Bank Management System

In order to facilitate and enhance the effectiveness of the day-to-day management of the INIBAP Transit Centre gene bank, an in-house developed searchable database system, *Musa* Gene Bank Management System (MGBMS), has been implemented recently. The application, developed in SQL 7.0 and Visual Basic 6.0, runs on Access 8.0 and is capable of processing all data at the accession level that are generated through the different gene bank operations, as well as related information. Reflecting the basic principles of gene bank management, the module attributes an identifier which is the unique gene bank accession ID number, called ITC code, assigned to the accession upon acquisition. This ID number is used by the system to link the passport data of the given accession, its designation status and taxonomic information, as well as additional information such as local names or uses of the accession, health status testing data and the availability of the accession for distribution. The system also keeps record of gene bank operation data including the storage location details, stocks and monitoring data of the accession in the active, base and lyophilized leaf collection and it manages germplasm orders and shipment processing data and contacts information.

Figure 2. Managing the inventory of the active *in vitro* collection at the INIBAP gene bank using a barcode based system.

As part of the MGBMS, bar-coding has been introduced in the *in vitro* gene bank to ensure accurate labelling of accessions. The use of a mobile device with integrated bar-code reader that communicates with the central database allows quick

collection and retrieval of accession information on site (Figure 2).

Musa Germplasm Information System

Scientists are unable to use crop germplasm unless and until they have easy access to detailed and accurate information on each accession. It is known that available information on banana genetic resources is scattered among numerous national and regional field collections or associated institutes. Moreover, as the quality and completeness of the available information varies widely among and within collections, its usefulness is limited.

In 1997, INIBAP laid the basis for a global information system for *Musa* through the release of MGIS (*Musa* Germplasm Information System) (Figure 3). The aim of the system is to enhance knowledge on *Musa* diversity, to help rationalizing conservation and to improve the use of banana genetic resources through a facilitated access to comprehensive information.

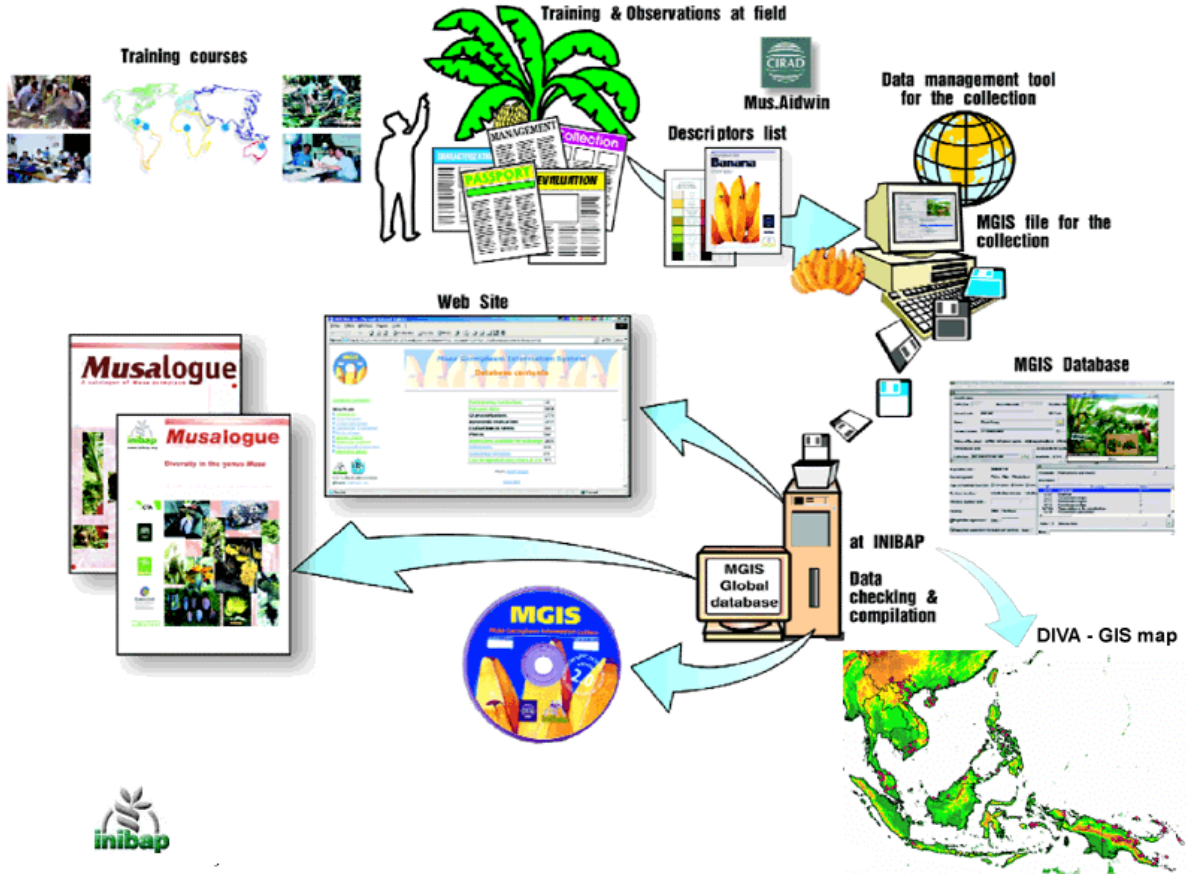

Figure 3. Structure of the *Musa* germplasm Information System (MGIS) and information flow through the system.

MGIS is based on a network of gene bank curators who have the primary responsibility of providing and entering data, following a standard format provided by the 'Descriptors list for bananas' (IPGRI-INIBAP/CIRAD, 1996). During specific training workshops, gene bank curators receive the MGIS CD-ROM to install locally an information management system for their proper collection. A tool is included in the system to send updated data through small binary files to IPGRI/INIBAP in Montpellier where all data are compiled, verified and fed into the central database.

Today, the MGIS database contains key information, including passport data, botanical classification, morpho-taxonomic descriptions and characteristics such as agronomic traits, disease resistance, stress tolerance, biochemical or molecular genetic markers, and plant

photographs as well as GIS information on 5188 accessions managed in 18 banana collections around the world making it the most extensive source of information on banana genetic resources. As more accessions will be included in MGIS, a more complete picture of the diversity managed in *ex situ* collections will emerge, based upon a broad range of standard information.

The database is publicly accessible through the internet (mgis.inibap.org or mgis.grinfo.net) and a PC-based version of MGIS containing updated collection information together with a stand-alone menu-driven software package is also made available on CD-ROM, upon request.

The global database can be queried on the identity, origin, characteristics and distribution of individual accessions in the collections. This allows curators of the participating institutions worldwide to share and compare their data. The database is also particularly helpful for various germplasm users namely breeders, researchers and farmer communities, in locating alternative sources of banana germplasm and identifying the most appropriate accessions with particular traits of interest.

From the data recorded in MGIS, two volumes of Musalogue, a catalogue of *Musa* germplasm, have been published. The first volume describes the germplasm collected in Papua New Guinea (Arnaud & Horry, 1997). The second one presents the diversity in the genus *Musa* and contains the main morpho-taxonomic characteristics and photographs of wild and cultivated bananas in the various sections, groups and the most important subgroups (Daniells *et al*., 2001).

IPGRI/INIBAP also provides information on the global *Musa* collection to SINGER, the System-wide Information Network for Genetic Resources of the CGIAR (Consultative Group on International Agricultural Research) linking the genetic resources databases of all CGIAR centres. These centres hold more than half a million samples of crop, forage and tree diversity which is of vital importance for food security and agricultural development. The SINGER database is accessible via Internet at singer.grinfo.net.

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