INIBAP's Mandate

The International Network for the Improvement of Banana and Plantain (INIBAP) was established in 1984 and has its headquarters in Montpellier, France. INIBAP is a nonprofit organization whose aim is to increase the production of banana and plantain on smallholdings by:

- initiating, encouraging, supporting, conducting, and coordinating research aimed at improving the production of banana and plantain;
- strengthening regional and national programs concerned with improved and disease-free banana and plantain genetic material;
- facilitating the interchange of healthy germplasm and assisting in the establishment and analysis of regional and global trials of new and improved cultivars;
- promoting the gathering and exchange of documentation and information; and
- supporting the training of research workers and technicians.

Planning for the creation of INIBAP began in 1981 in Ibadan with a resolution passed at a conference of the International Association for Research on Plantain and Bananas. In May 1994, INIBAP was brought under the governance and administration of the International Plant Genetic Resources Institute (IPGRI) to enhance opportunities for serving the interest of small-scale banana and plantain producers.

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Cover illustration: Symptoms of black leaf streak/black Sigatoka disease on a leaf of a highly susceptible 'Cavendish' cultivar growing on Aitutaki Island, Cook Islands (photo: DR Jones, INIBAP).

The Improvement and Testing of *Musa*: a Global Partnership

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INTERNATIONAL NETWORK FOR THE IMPROVEMENT OF BANANA AND PLANTAIN
inibap

Musa Germplasm Distribution from the INIBAP Transit Center

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Introduction

The INIBAP Transit Center (ITC), located at the Catholic University of Leuven (KUL), Belgium, holds in trust the largest in-vitro *Musa* collection in the world. Besides its role as a gene bank, the ITC is also involved in the international transfer of banana genetic resources, via the *Musa* Germplasm Exchange System (MGES), to plant breeders and plant researchers throughout the world. The ITC also plays a key role in the International *Musa* Testing Program (IMTP), providing selected germplasm to ecologically different testing sites. As part of the activities of the *Musa* Germplasm Information System (MGIS), the ITC is to furnish a standard set of *Musa* germplasm accessions to institutes interested in participating in taxonomic studies.

ITC started its activities in 1985 with a core collection of 17 accessions. Its collection now contains 1056 accessions (April 1994), representing the large genetic diversity within the genus *Musa*. The accessions were acquired from curators of other existing collections in the world, breeding programs, NARSs, research workers, botanical gardens, and collecting missions in 38 different locations.

Medium-term Storage

During the early 1980s, tissue-culture techniques for rapid clonal propagation and storage under limited growth conditions were investigated at the KUL Laboratory of Tropical Crop Husbandry (Banerjee, De Langhe 1985). From this work, standard protocols were developed which are outlined below.

Proliferating tissue cultures are maintained on a Murashige and Skoog (1962) mineral salt mixture, supplemented with 10 µM (2.25 mg.L^{-1}) N6-benzylaminopurine (BA), 1 µM (0.175 mg.L^{-1}) indole-3-acetic acid (IAA), Murashige and Skoog vitamins, 30 g.L^{-1} sucrose, 10 mg.L^{-1} ascorbic acid and 2 g.L^{-1} gelrite. The pH is adjusted to 5.8 before autoclaving for 20 min at 120°C. A relatively high level of cytokinin is used to reduce the dominance of the apical meristem with the result that adventitious shoots and buds arise from the explant.

Under normal growth conditions (28 ± 2°C and 5000 lux), proliferating tissues of shoot tips need to be subcultured every 6-8 weeks. At the ITC, slow growth is achieved by storing cultures at a temperature of 15 ± 1°C and a light intensity of 2000 lux. Temperatures below 14°C cause damage and subsequently provoke serious losses.

Under slow growth conditions, accessions are subcultured only once per year on average (De Smet, Van den houwe 1991). Some accessions, however, can be stored up to a maximum of nearly 615 days, while others need to be subcultured every 60 days (De Smet et al., submitted for publication). These large differences in storage capacity are related to genomic composition: for example, East African highland banana cultivars (AAA) and AAB banana types (other than plantains) can be stored significantly longer than all other genotypes. Also, in general, parthenocarpic bananas can be stored for longer periods than wild bananas. In particular, storage time for wild *Musa balbisiana* accessions is significantly shorter than for any other genotype (De Smet et al., submitted for publication).

Germplasm Distribution

Since 1985, the ITC has distributed accessions held in the collection to interested research institutes and plant breeders. So far, the ITC has exported more than 2500 accessions, which means that, on average, one accession is supplied per working day. The number of accessions supplied has increased, especially since 1993 (Table 1). To date, nearly 65 institutes from all continents have benefited from this activity. To minimize the spread of economically important pests and diseases, all operations involved in the shipment of germplasm follow, in essence, the FAO/IBPGR/INIBAP Technical Guidelines

Table 1. Export of *Musa* germplasm from the INIBAP Transit Center for the period 1985-93.

	1985	1986	1987	1988	1989	1990	1991	1992	1993	Total	%
Latin America and Caribbean	0	0	13	127	88	111	68	61	99	567	24
West and Central Africa	20	27	154	105	100	54	79	0	24	572	24
East Africa	0	0	0	105	69	42	21	48	66	351	15
Asia and Pacific	12	0	3	5	48	68	50	83	75	344	14
Europe	0	0	10	14	34	122	103	104	179	566	24
TOTAL	32	27	180	356	348	397	321	296	443	2400	100

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for the Safe Movement of *Musa* Germplasm (Frison, Putter 1989). This document provides several recommendations for the transfer of *Musa* germplasm.

Proliferating tissue cultures (Fig.1)

If the person ordering germplasm has access to an in-vitro laboratory and micropropagation is possible, samples of proliferating tissue cultures are provided. The ITC selects proliferating cultures from the clone stored under medium-term storage conditions and subcultures it on a new proliferation medium if necessary. After approximately 5 weeks of growth under normal growth conditions, the cultures are trimmed and transferred into plastic culture vessels containing 15 mL of proliferation medium. Cultures are then grown under normal growth conditions for 2 weeks. Seven proliferating cultures are prepared per accession and the five best-performing cultures are selected for dispatch. Each individual culture vessel bears a label with the ITC code and the accession name, and is packed in shock-absorbing watertight material. Cultures are dispatched from the ITC about 2 months after the order is received from INIBAP headquarters (Table 2).

Rooted plantlets (Fig.1)

If in-vitro facilities are not available to the person ordering the germplasm, the ITC supplies rooted plantlets. The time needed to fulfill an order for rooted plantlets is about 4 months on average (Table 2). The most suitable germplasm for this purpose is identifiable in culture as a cluster of 5-10 shoots on proliferation-inducing medium. This material can be easily multiplied to a high number of cultures and the shoots can be easily separated from each other for regeneration into individual plantlets. On a regeneration medium, these shoots will still produce a few buds or tiny shoots at their bases, but these can be removed during subculture.

Many cultures grow differently and this seems to be dependent on genotype. For example, East African highland banana cultivars and wild *Musa acuminata* species form a single shoot or a cluster of a few shoots in vitro. Hence their regeneration is fast, but their proliferation slow. The degree of proliferation increases when the portion of the B-genome in the genotypic constitution increases. ABB and BB accessions, therefore, multiply very fast, forming clusters of meristems covered with small leaves. However, their regeneration to single plantlets is very time-consuming. Experience has shown that three to six subcultures on a regeneration medium are required to obtain individual rooted shoots of ABB and BB clones. This takes about 6-8 months. The blackening of the culture, which is related to a high level of proliferation, is in addition a hindering factor for the regeneration of plantlets. ABB and BB accessions show considerably more oxidation of polyphenolic compounds than accessions belonging to other genotypes.

Cultures, even within one genotype, range from one shoot to a cluster of shoots. This is probably due to the random selection of the explants (apical meristems and adventitious buds) during subculturing. This heterogeneous growth response thus prolongs the time to supply an adequate amount of homogeneously growing plantlets.

After selection of proliferating cultures under medium-term storage conditions, the accessions are either subcultured once or a few times on a regeneration medium. This

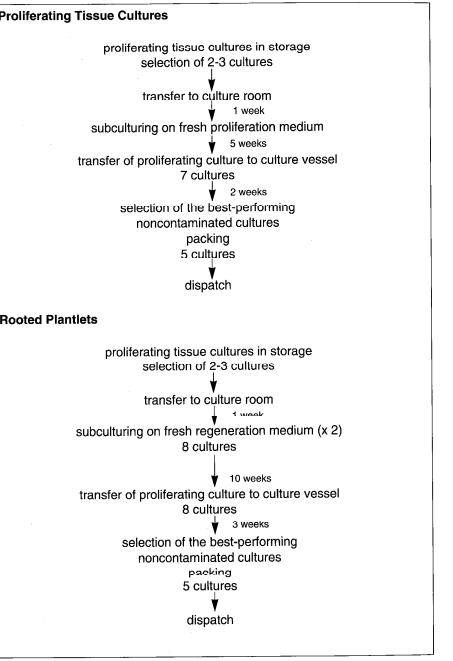

Figure 1. Preparation of *Musa* germplasm for distribution.

Table 2. Duration in months between receiving a request for germplasm and dispatch.

Proliferating tissue cultures				
Year	Type of vial	Average	Maximum	Minimum
before 1992	Plastic culture vessel	2.2	6.8	0.5
1992	Plastic culture vessel	2.2	6.5	0.5
1993	Plastic culture vessel	2.1	4.5	0.5
Rooted plantlets				
Year	Type of vial	Average	Maximum	Minimum
before 1992	Glass test-tube	3.2	6.8	0.5
1992	Cultu sak®	3.8	12	0.5
1992	Cultu sak®	3.9	10	0.5

regeneration medium differs from the proliferation medium in cytokinin content, which is reduced to 1 µM. Eight regenerated shoots are transferred into sterile Cultu saks® filled with 10 mL of rooting medium (i.e. a Murashige and Skoog [1962]) mineral salt mixture at half strength, supplemented with 1 µM (0.203 $mg.L^{-1}$) of the stronger auxin indole butyric acid (IBA) to induce rooting, Murashige and Skoog vitamins, 30 $g.L^{-1}$ sucrose, 10 $mg.L^{-1}$ ascorbic acid, and 2 $g.L^{-1}$ gelrite. Plantlets about 4 cm tall and with three to four leaves are most suitable for transferring to Cultu saks®. For 3 weeks they are kept in the culture room in order to grow and develop roots. The five best-performing plantlets are carefully selected for dispatch. Each individual bag has a label bearing the ITC code and the accession name.

Early in 1992, the ITC switched from using glass tubes to Cultu saks® for the dispatch of rooted plantlets. They are a valuable alternative to glass tubes because they have the positive attributes of being airtight and watertight. They are flexible and able to withstand shock during transportation. In addition, they protect the culture from contaminants, but allow gas exchange. Upon arrival, plantlets that are 5-10 cm tall and have a well developed root system can be planted out in soil in a nursery. If the plantlets are smaller, or if transplanting is not immediately possible, it is advisable to keep the plantlets in the Cultu saks® in an upright position under sufficient light, but not direct sunlight, at temperatures between 20 and 30°C. Experience has shown that such cultures grow and can be kept for at least 8 weeks under these conditions.

Shipments of *Musa* germplasm from the ITC to clients are always accompanied by a letter and a packing list. A questionnaire on the condition in which the material arrived at its destination (receiving report) is also enclosed. This is completed by the receiver and returned to the ITC. A phytosanitary certificate, issued by the plant quarantine service of the Belgian Ministry of Agriculture, accompanies the exported material together with a commercial invoice for countries outside the European Union. From some recipient countries, an import permit is required before material can be sent.

Recently, shipments of rooted plantlets have also been accompanied by recommendations on how to handle these young in-vitro plants after deflasking.

All germplasm is shipped by courier and reaches its destination within 1 week after dispatch from the ITC.

Distribution of Germplasm for IMTP

As a part of the IMTP Phase I, the ITC produced and distributed about 1500 rooted plantlets to six ecologically different testing sites: CORBANA (Costa Rica), CRBP (Cameroon), FHIA (Honduras), ICA (Colombia), IITA (Nigeria), and IRAZ (Burundi).

Seven hybrids from FHIA were selected for evaluation for resistance to black leaf streak/black Sigatoka disease and nine standard host-range accessions were also tested. The distribution of germplasm started in December 1990 and, by the end of 1991, all testing sites had received the entire set of accessions. The germplasm was sent as 15 rooted plantlets per accession which were individually packed in glass test-tubes.

Many accessions selected for IMTP Phase II were only received from donor institutes in 1993. Before distribution to the different testing sites proceeds, all accessions involved will be indexed at an INIBAP Virus Indexing Center (VIC). Between 24 February 1993 and 7 October 1993, five plantlets of the accessions involved were regenerated and sent to the VICs in either France (VIC-CIRAD) or Australia (VIC-QDPI).

In 1993, the ITC started the multiplication of proliferating cultures of the relevant accessions for IMTP Phase II. These stock cultures are stored under reduced growth conditions awaiting final virus indexing results. When the results are known, the ITC will start doubling the desired number of stock cultures.

Proliferating tissue cultures will be sent to those collaborators who have facilities for in-vitro culture and who can produce their own plantlets (Table 3). The shipments will begin late in 1994.

The ITC will also deliver about 9 240 sterile rooted plantlets, individually packed in Cultu saks®. For the Sigatoka trial, 11 accessions are involved and 35 plantlets of each will be dispatched to every site. There will be 6 test sites. The Fusarium wilt trial is larger as 21 accessions and 11 testing sites need plantlets (Table 3). Thirty plantlets of each accession will be sent to each site.

The ITC is to furnish stock cultures to a private tissue culture laboratory. This laboratory will produce and pack sterile rooted plantlets and deliver them to the ITC. They will be checked for contamination, labelled, and dispatched to the test sites. The first shipments of plantlets for IMTP Phase II are planned for early 1995.

All interested parties will be informed of the date and details of the shipment and in some cases the receiving institutes will be requested to provide the ITC with an import permit 3 months before the planned date of dispatch. Collaborators have indicated to the ITC when it would not be appropriate to receive plantlets because of adverse planting conditions.

Table 3. IMTP Phase II collaborators and their requirements for either proliferating tissue cultures or plantlets.

Sigatoka sites	
Proliferating tissue cultures:	Cameroon (CRBP[1]) Costa Rica (CORBANA) Cuba (INISAV) Honduras (FHIA) India (ICAR) Nigeria (IITA)
Plantlets:	Colombia (ICA) Philippines (BPI) St. Lucia (WINBAN) Thailand (HRI) Tonga (MAFF) Uganda (NARO)
Fusarium wilt sites	
Proliferating tissue cultures:	Cuba (INISAV) Honduras (FHIA) India (ICAR) South Africa (BPIU)
Plantlets:	Australia (QDPI) Brazil (CNPMF-EMBRAPA) Canary Islands (CITA) Indonesia (AARD) Malaysia (MARDI) Philippines (BPI) Taiwan (TBRI) Thailand (HRI) Uganda (NARO)

[1] see list of acronyms and abbreviations on page 287

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