Preparation of slides for chromosome studies in Musa spp.
Do natural AxB tetraploid bananas exist?
Chemical variability in the genus Musa
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Propagation and diffusion of improved banana varieties in Tanzania
Musa in multistrata systems
Selection of plant type for banana nematode sampling
Black Sigatoka disease control in Cuba

Black Sigatoka confirmed in Brazil
Update on black Sigatoka in Venezuela
Books etc.
MusaForum
INIBAP news
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The mission of the International Network for the Improvement of Banana and Plantain is to increase the productivity and stability of banana and plantain grown on smallholdings for domestic consumption and for local and export markets.

INIBAP has four specific objectives:

- to organize and coordinate a global research effort on banana and plantain, aimed at the development, evaluation and dissemination of improved cultivars and at the conservation and use of Musa diversity;
- to promote and strengthen regional efforts to address region-specific problems and to assist national programmes within the regions to contribute towards, and benefit from, the global research effort;
- to strengthen the ability of NARS to conduct research on bananas and plantains;
- to coordinate, facilitate and support the production, collection and exchange of information and documentation related to banana and plantain.

Since May 1994, INIBAP is a programme of the International Plant Genetic Resources Institute (IPGRI).

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A novel method to prepare slides for high resolution chromosome studies in Musa spp.

Jaroslav Doležel, Marie Doleželová, Nicolas Roux and Ines Van den houwe

Contrary to the importance of bananas and plantains both as a staple food and export commodity, the knowledge of their genome at the chromosomal level remains poor. It is surprising that most genotypes maintained in gene banks have unknown, a speculative or even a wrongly determined ploidy level (Enny et al. 1997, Horry et al. 1998). Although ploidy can be now determined also by flow cytometry (Doležel et al. 1994, 1997) detailed microscopical analysis of karyotype cannot be replaced by other methods. In contrast with the situation in other economically important crops, the state of Musa karyology is rather poor. This is mainly due to problems with preparation of chromosome spreads and the small size of Musa chromosomes (1 - 2 µm, when observed at mitotic metaphase). Considering the nuclear genome size in Musa (Doležel et al. 1994) an average chromosome has only about 50 Mbp DNA.

Difficulties in preparing good metaphase spreads are mainly due to the rigid cell wall. The tissue can be softened by maceration in strong acids or by incubation in hydrolytic enzymes, and metaphase spreads can be obtained by squashing the softened tissue. In Musa, several authors used this technique and in some cases, metaphase spreads suitable for chromosome counting were obtained (Shepherd and Dos Santos 1996, Osuji et al. 1998). However, due to the presence of remnants of cell wall and cytoplasm, the squash technique is not suitable for high resolution chromosome analysis as the cytoplasm may be non-specifically stained. Furthermore, squash preparations are not optimal for physical mapping of DNA sequences using in situ hybridization. The presence of cellular remnants decreases the accessibility of chromosomes to molecular probes and may also cause their non-specific binding. Alternative procedures based on protoplast isolation and spreading were developed for some species to avoid this problem (Ma et al. 1996, Martin et al. 1994). This paper describes a protocol for the preparation of high quality metaphase spreads for chromosome analysis in Musa. The protocol is based on protoplasts isolation from root tips and was developed through many experiments in which each step of the procedure was optimized.

Materials and methods

All Musa species and clones used in this study (Table 1) were obtained from the INIBAP Transit Centre (Katholieke Universiteit Leuven, Belgium) as in vitro rooted plantlets. After transfer to soil, plants were maintained in a greenhouse.

Actively growing roots were cut about 1 cm from the root tip and collected in 50 mM phosphate buffer (pH 7.0) containing 0.2% β-mercaptoethanol. The roots were then transferred to 0.05% 8-hydroxyquinoline for three hours at room temperature to accumulate dividing cells in metaphase. Subsequently, the roots were fixed in 3:1 (ethanol: acetic acid) fixative at 4°C overnight. After two rinses in 70% ethanol, the roots were transferred to 70% ethanol and stored at 4°C for up to several months.

Prior to slide preparation, 5 to 15 roots were washed three times in a solution of 75 mM KCl and 7.5 mM EDTA (pH 4). Meristem tips were then cut to small pieces and digested for 60 min at 30 °C in 400 µl of enzyme mixture in a microcentrifuge tube. The enzyme mixture consisted of 1% pectinase (Sigma P-2401), 0.5% pectolase (Sigma P-3026) and 0.5% cellulase (Serva 16419) made in 0.1M citrate buffer (pH 4.7). The suspension of released protoplasts was filtered through a 150 µm nylon mesh and pelleted at 1000 rpm. The pellet was resuspended in 400 µl of 75 mM KCl and 7.5 mM EDTA (pH 4) and incubated for 5 min at room temperature. After pelleting, the protoplasts were resuspended in 400 µl of ice-cold 70% ethanol, incubated for 5 min and pelleted again. After resuspending in ice-cold 70% ethanol, isolated protoplasts were stored at 4 °C for up to several months.

To prepare a slide, 7 µl of protoplast suspension in 70% ethanol was dropped onto a clean ice-cold microscope slide. The suspension was allowed to spread out and air-dry. This process was monitored under a microscope. Shortly before complete drying out, 7 µl of ice-cold 3:1 fixative was added to the drop to induce cell bursting. Shortly before complete drying the slide was briefly rinsed in 100% ethanol and air-dried at room temperature. For chromosome staining, the slides were immersed for 25 min at room temperature in 3% Giemsa solution made in 50 mM phosphate buffer. The stain was removed by a wash in distilled water. Air-dried slides were mounted in Euparal and observed under Olympus BX60 microscope using a 100x/1.35 oil immersion objective. Images were photographed.

Figure 1. Mitotic metaphase plates of selected Musa genotypes: A ‘Niyarma Yik’ (ITC0269); 2n=2x=22 chromosomes; B ‘Balonkawe’ (ITC0473); 2n=3x=33 chromosomes; C ‘Wisang Janbe’ (ITC0694); 2n=3x=33 chromosomes; D ‘(Kluai) Ngoen’ (ITC0286); 2n=4x=44 chromosomes. Bar = 10 µm.
on Ilford PAN F 50 film using a green optical filter.

Results and discussion
After putting on a slide, the protoplast suspension in 70% ethanol remains in a drop without significant spreading. When the fixative (3:1) is added, the drop spreads on the glass surface and begins to evaporate rapidly. As the mixture evaporates, the surface tension makes the protoplasts wider from side to side and eventually causes the protoplasts to break. At this moment the metaphase plate is released from the protoplast, the cytoplasm is dispersed and chromosomes spread. We have found that the quality of metaphase spread depends critically on the rate of drying. If the drying is too fast, the protoplasts dry without sufficient spreading and metaphases are clumped. On the other hand, very slow drying may lead to broken metaphases. Optimal conditions for drying have to be found empirically. The rate of drying can be controlled by the temperature of the microscope slide, the fixative and (to a certain extent) by room temperature. Although the method described here is more laborious than traditionally used procedures, it has important advantages. Pre-treated roots and even isolated protoplasts can be stored in a freezer and used for plant regeneration. To-pre-treated roots and even isolated protoplasts can be stored in a freezer and used for plant regeneration.

Table 1. Determination of chromosome number in selected Musa species and landraces.

<table>
<thead>
<tr>
<th>Accession name</th>
<th>ITC Code*</th>
<th>Species/ Group</th>
<th>Subspecies/ Sub-group</th>
<th>Chromosome number</th>
<th>Ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higa</td>
<td>0428</td>
<td>acuminata</td>
<td>banksii</td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>Pisang Mas</td>
<td>0653</td>
<td>AA</td>
<td>Sucrier</td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>Pa (Rayong)</td>
<td>0672</td>
<td>acuminata</td>
<td>siamea</td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>Niyarma Yik</td>
<td>0269</td>
<td>AA</td>
<td>banksi - derivative</td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>M. balbisiana</td>
<td>0094</td>
<td>balbisiana</td>
<td></td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>Cameroun</td>
<td>0246</td>
<td>balbisiana</td>
<td></td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>Singapuri</td>
<td>0248</td>
<td>balbisiana</td>
<td></td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>Tani</td>
<td>1120</td>
<td>balbisiana</td>
<td></td>
<td>22</td>
<td>2x</td>
</tr>
<tr>
<td>Yangambi km5</td>
<td>1123</td>
<td>AAA</td>
<td>Ibota</td>
<td>33</td>
<td>3x</td>
</tr>
<tr>
<td>Obino ‘E’Wai</td>
<td>0109</td>
<td>AAB</td>
<td>Plantain (French)</td>
<td>33</td>
<td>3x</td>
</tr>
<tr>
<td>Agbagba</td>
<td>0111</td>
<td>AAB</td>
<td>Plantain (False Horn)</td>
<td>33</td>
<td>3x</td>
</tr>
<tr>
<td>Saba</td>
<td>1138</td>
<td>ABB</td>
<td>Saba</td>
<td>33</td>
<td>3x</td>
</tr>
<tr>
<td>Klui Tiranot</td>
<td>0652</td>
<td>?</td>
<td>Klui Teparad</td>
<td>33</td>
<td>3x</td>
</tr>
<tr>
<td>Balonkawe</td>
<td>0470</td>
<td>?</td>
<td>Klui Teparad</td>
<td>33</td>
<td>3x</td>
</tr>
<tr>
<td>Pisang Jambo</td>
<td>0694</td>
<td>?</td>
<td></td>
<td>33</td>
<td>3x</td>
</tr>
<tr>
<td>(Klui) Ngoen</td>
<td>0286</td>
<td>AABB</td>
<td></td>
<td>44</td>
<td>4x</td>
</tr>
</tbody>
</table>

*Code assigned by the INIBAP Transit Centre (Leuven).

makes chromosome counting and the analysis of chromosome morphology at metaphase or prometaphase very easy. We have found that slides prepared according to this method are suitable for physical mapping of DNA sequences on Musa chromosomes using in situ hybridization (Doležel 1996, Doleželová et al. 1997).

The method has been tested on a random selection of genotypes representing Musa acuminata, M. balbisiana, a range of triploid clones and one tetraploid clone (Table 1). In all cases, chromosome numbers could be unambiguously determined. Our results confirmed the reclassification of ‘Kluai Tiranot’ as a triploid clone (Jenny et al. 1997). Contrary to other reports indicating the occurrence of chromosome number instability in Musa (Sandoval et al. 1996, Shepherd and Da Silva 1996), our results obtained on a limited number of plants did not indicate a wide-spread occurrence of aneuploidy or mixoploidy in these genotypes. Furthermore, high quality of metaphase spreads permitted evaluation of chromosome morphology. To conclude, the procedure described here opens a way for reliable chromosome counting in Musa, for high resolution studies of their morphology and for physical mapping of Musa genome using in situ hybridization.

Acknowledgements
We thank O. Blahousék, M.Sc, for assistance with microphotography and Dr W. Busch (Munich, Germany) for useful suggestions during the course of this work. This study was undertaken as a part of the Global Programme for Musa Improvement (PROMUSA) and was supported in part by the Research Contract No. 8145/RB from the International Atomic Energy Agency, Vienna, Austria.

References


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Do natural AxB tetraploid bananas exist?

Jean-Pierre Horry, Jaroslav Doležel, Marie Doleželova and Martin A. Lysak.

In 1997, Jenny and co-workers published one of the most astounding results in the area of banana taxonomy: the clone considered as the reference for natural tetraploid bananas and which is well known throughout the world as 'Klue Teparod' or 'Tiparot' (ABB'B) is in fact a triploid (Jenny et al. 1997). Initially such a finding, contrasting so strongly with unanimously accepted fact, puzzled the authors. However, they could not find the flaw in their research. Thailand is the recognised country of origin of 'Tiparot', a corrupted form of the Thai name 'Kluai Thephphepharot', which means 'banana of divine taste'. The accession evaluated was introduced as 'Klue Teparod' (accession No. THA 20) into the CIRAD genebank directly from Thailand. Its morphological description fully agrees with the previous descriptions of the clone. Chromosome counting was independently assessed using two methods (flow cytometry and classical microscopy) and molecular analysis through RFLP corroborated the result. Moreover, in tracing the origin of the classification of 'Tiparot' as a tetraploid, the authors did not find any evidence of a chromosome count of 44 in the published literature. The tetraploid nature of the clone seems to rest on an assumption based on the pioneering works of White (1928) rather than on direct evidence. "...P.R. White (1928) found two clones bearing 40 chromosomes (the count may have been too low by four chromosomes) [...] One of these, a Philippine variety 'Tiparot', was undoubtedly the clone referred to by Simmonds and Shepherd (1955) ..." (Richardson et al., 1965). A detailed examination of White's 1928 publication shows that chromosome numbers were more often underestimated rather than underestimated, actual Munusa diploids samples being counted as 24, triploids as 32 to 36. In view of this fact, we decided to investigate further the ploidy of some supposed tetraploids held in the INIBAP genebank.

Two independent methods were employed to determine their ploidy: karyological analysis to establish chromosome number in root-tip cells and flow cytometry to estimate nuclear DNA content in leaf cells. Preparation of nuclei suspensions for flow cytometry was performed as described by Doležel et al. (1994, 1997). Fluorescence of DAPI-stained nuclei was then analysed using a Partec PAS flow cytometer which was calibrated so that a G1 peak of a diploid control (Pisang Mas, ITC0653) was on channel 100. In each accession, four plants were analysed, each of them five times. Chromosome counting was performed on slides prepared according to a novel method for preparation of chromosome spreads in Musa (Doležel et al. 1998). The slides were evaluated using an Olympus BX60 microscope at a 1000x magnification. In each accession, slides from four plants were analysed, on each slide at least 20 metaphases or prometa-phases were counted.

The same accession THA 20, introduced into the INIBAP genebank as ITC0652 in 1989, was analysed again in this study at the Institute of Experimental Botany and both flow cytometry and chromosome counting confirmed unambiguously its triploid (2n = 3x = 33) status (Figures 1, 2A). Moreover, the analysis clearly showed that the accession 'Balonkawe' (ITC0473), which is recognised as a synonym of 'Tiparot', is also triploid (2n = 3x = 33, Figure 2B). This accession was reported as a tetraploid in the publication of Richardson et al. (1965), and was introduced to the ITC from FHIA. The clone was originally introduced into Honduras from the Philippines as II-42 by the United Fruit Company in the early 1960s.

Thus, contrary to the general belief, all available evidence indicates that it is wrong to continue to consider 'Klue Teparod' as a tetraploid and its triploidy should be generally accepted.

Another two clones, 'Pisang Jambe' and '(Kluai Ngoen', which came to CIRAD from Indonesia and Thailand respectively in 1986, and were passed thence to the INIBAP collection in 1989 were also analysed. 'Pisang Jambe' (ITC0694), classified as an AAAA tetraploid from its morphological behaviour, was found to be triploid (2n = 3x = 33, Figure 2C). '(Kluai Ngoen' (ITC0286) for its part, was described in Malaysia as a triploid...
AAB cooking banana (Chomchalow and Silayoi 1984). However, field observations from the CIARD researchers led them to reconsider this position and to re-classify it as an AABB tetraploid clone. Results of both flow cytometry and chromosome counting in root tips confirmed its tetraploid status (2n = 4x = 44, Figure 2D).

Since the time when Simmonds and Shepherd wrote that the only naturally occurring tetraploid banana known is 'Klue Teparod'(Simmonds and Shepherd 1955), several other clones have been considered as natural tetraploids (Stover and Simmonds 1987). Besides the 'Klue Teparod' "ABB" group, the authors refer to other tetraploids assigned to AAAA, AAB, AAB or ABBB groups. A common feature of all these accessions is their origin: Papua New Guinea and the surrounding islands. Carreel (1994) examined through RFLP some tetraploid varieties introduced from the same area and found that these tetraploids are not pure Eumusa derivatives, but are introgressed with Australimusa genome. Could it be that the above mentioned tetraploids result from the same kind of introgression? And if the answer is yes, are natural acuminata x balbisiana tetraploid cultivars even more rare than earlier suggested? This question will remain open until the genome content of the tetraploid clones is properly evaluated at the molecular level. Not all of the supposed tetraploid clones have been properly analysed and it is likely that some mistakes might still persist. In the light of these examples, it is clear that one cannot rely on the morphology of the plant alone to determine its classification. Fortunately, new or improved methods including chromosome counting and flow cytometry are now available and thus the ploidy of all suspected accessions may be reanalysed rapidly and with sufficient reliability.

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References


Chemical variability in the genus Musa:
Genetic characterization using nine enzyme systems

Musa species and cultivars are identified mainly on the basis of morphological characters. However, the genetic links between cultivars and somatic mutations and morphological changes caused by the environment are major obstacles to the accurate identification of clones (Kaemmer et al. 1992). Isoenzymatic markers have been widely used in Musa in various research frameworks and by different groups of researchers (Horry 1989, Espino and Pimentel 1990, Jarret and Litz, 1986a, Bhat et al. 1992a, 1992b, Lebot et al. 1993).

The main objective of this work was the genetic characterization of 15 clones in the Colombian Collection of Musaceae (CCM) by using isoenzymatic markers to facilitate subsequent analysis of the genetic variability in the CCM.

Material and methods
A selection of 15 accessions of various genome groups derived from the CCM (El Agradable experimental province, Quindío province) were cultured in vitro at the CORPOICA research centre at Tibaitata (Table 1). A 0.5g tissue fragment taken from the centre of the leaves of plantlets cultured in vitro was placed on cold, moistened White P.R. 1928. Studies on the banana. An investigation of the floral morphology and cytology of certain types of the genus Musa L. Zelts. Für Zellforschung und mikroskopische Anatomie 7:673-733.
filter paper. The sample was ground in liquid nitrogen with 1.5 ml extraction buffer (Tris-HCl 0.1 M, pH 7.5; PVPP 5% Sucrose 10% DTT 10 mM; Triton X-100 at 0.1% and 2-mercaptoethanol 14 mM, according to Beltrán et al. 1996). After centrifugation (5500 rpm for 30 min), the supernatant was collected in 250 μl aliquots and stored at −70°C.

Polyacrylamide gels were prepared according to the method of Bhat and Lakhanpaul (1995). Three migration systems were used according to the enzymes sought (Table 2). The migration indicator consisted of a trace of bromophenol blue diluted in 1 ml extraction buffer. For band visualization, the gels were stained using the procedures described for other species by Shaw and Prasad (1970), Stuber et al. (1988), Hollis et al. (1983) and Vallejos (1983), with a few modifications (Table 3). After staining, the gels were washed and fixed in glacial acetic acid, methanol and water solution (1:6:14).

Once fixed, the gels were interpreted for determination of zones of activity (loci) and the number of bands per locus (alleles) for the enzymes tested. The locus closest to the anode is numbered 1, the next is 2 and so forth; for each locus, the alloenzyme that has migrated most rapidly is called a, the second is b and so forth.

Results and discussion

Nine enzymes among the 23 enzymatic systems evaluated displayed interesting bands. The diagrammes observed for the enzymes EST, PGM, SKDH, MDH, DIA, ME, PGDH, RUB and PRX are shown in Figures 1 and 2. RUB and PRX are monomorphic in all the accessions studied.

Esterases (EST): a total of 14 bands corresponding to five zones of enzymatic activity were revealed for the 15 clones analysed. The zone closest to the anode, Est-1, revealed two monomeric alleles in all the clones. Four alleles were present in the second zone, Est-2. Two separate bands for the heterozygote phenotype of the diploid clones suggest that the enzyme is monomeric. Zone Est-3 displayed three alleles distributed in all the material. Zones Est-4 and Est-5 displayed two and three alleles respectively: one band is visible in the case of homozygosity and two bands in heterozygosity. This leads us to concluding that these isozymes have a monomeric structure (Figure 1).

Jarret and Litz (1986b and 1986c) describe 30 and 14 bands respectively, indicating variation resulting from the presence of minor bands related to the physiological state of the plant.

The esterases were the most polymorphic system for the clones studied (Table 4). The genome group AAA displayed the greatest variability. In a total of 14 alleles, the clones ‘Cavendish’ and ‘Red’ displayed 11, with the intermediate zone Est-3 being the most polymorphic. Several authors have stressed the high degree of polymorphism revealed by this system with regard to both the number of loci and the number of alleles detected per locus. In the case of Musa, it has been presented as an isozyme whose use might be possible in clonal identification, although the link between the band diagrammes and the genome group is not perfectly clear (Bassiri and Adams 1978, Stuber et al. 1988, Jarret

Table 2. The migration systems used (polyacrylamide gel electrophoresis).

<table>
<thead>
<tr>
<th>System</th>
<th>Enzyme (1)</th>
<th>Concentration</th>
<th>Separation gel</th>
<th>Migration buffer</th>
<th>Migration conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Jarret and Litz 1986b)</td>
<td>EST, PRX</td>
<td>Tris HCl 0.065 M pH 6.7 Acrylamide 4%</td>
<td>Tris HCl 0.375 M pH 8.8 Acrylamide 8%</td>
<td>Tris 0.005 M Glycine 0.0384 M pH 8.3</td>
<td>50V - 15’ 100V - 15’ 150V - 30’ 200V - 15’ 250V - final 200V - Final</td>
</tr>
<tr>
<td>II (Bhat et Lakhanpaul 1995)</td>
<td>MDH</td>
<td>Tris HCl 0.375 M pH 8.9 Acrylamide 4.6%</td>
<td>Tris HCl 0.375 M pH 8.9 Acrylamide 7.7%</td>
<td>Tris 0.02 M Glycine 0.19 M pH 8.8</td>
<td>70V - 60’ 190V - Final</td>
</tr>
<tr>
<td>III (Hollis et al. 1986)</td>
<td>DIA, ME, PGM, PGDH, SKDH, RUB</td>
<td>Tris HCl 0.5 M pH 6.8 Acrylamide 4%</td>
<td>Tris HCl 1.5 M pH 8.8 Acrylamide 8%</td>
<td>Tris 0.06 M Ac.borique .017 M pH 8.8</td>
<td>50V - 15’ 100V - 15’ 150V - 30’ 200V - 15’ 250V - final</td>
</tr>
</tbody>
</table>

(1) EST = Esterase, PRX = Peroxidase, MDH = Malate dehydrogenase, DIA = Diaphorase, ME = Malic enzyme, PGM = Phosphoglucomutase, PGDH = Phosphogluconate dehydrogenase, SKDH = Shikimate dehydrogenase, RUB = Rubisco

Figure 1. Isoenzymatic profiles of EST, PGM, SKDH and MDH for the genotypes studied (anode migration).

Figure 2. Isoenzymatic profiles of DIA, ME, PGDH and PRX for the genotypes studied (anode migration).

**Phosphoglucomutase (PGM):** This enzyme displays two zones of activity, Pgm-1 and Pgm-2, with three bands for each. The presence of one band for homozygotic individuals and two bands for heterozygotes suggests that the enzyme has a monomeric structure at each of the two loci (Figure 1). This is in agreement with the conclusions of other authors concerning both Musa (Horry 1989, Lebot et al. 1993) and other species: maize (Stuber et al. 1988) and sweet potato (Reyes and Collins 1992).

Lebot et al. (1993) identified three zones of enzymatic activity on starch gel for Musa clones from Asia and the Pacific.

According to Jarret and Litz (1986b, 1986c), this system may reveal several zones of activity. Nevertheless, Pgm-1, the most anodic zone, has been described as the most conclusive as it enables the clear visualization of the bands in polyacrylamide gel. This is in agreement with our results.

The ABB clones display a large number of alloenzymes (five), distributed among the four clones tested. The AA and AAA clones generally displayed one allele in the Pgm-1 zone of activity and three alleles in zone Pgm-2 (Table 4). Shikimate dehydrogenase (SKDH): two zones of activity were detected, each with three alleles (Figure 1). This agrees with the results of Jarret and Litz (1986b, 1986c), Bhat et al. (1992b), Espino and Pimentel (1990) and Horry (1989). These authors observed five mobile alleles and two loci coding SKDH.

ABB triploid zones display considerable variability, with observation of five of the six alleles observed (Table 4). The two diploid clones display the same two zones of enzymatic activity as the triploid clones.

**Malate dehydrogenase (MDH):** A single zone of activity was revealed in all the experiments, Mdh-1. Our observations confirm the dimeric structure of the enzyme reported by Jarret and Litz (1986b, 1986c). Bhat et al. (1992b) report a high degree of polymorphism in this enzyme, with four zones of enzymatic activity. Three alleles were observed in the AAA clones, including the ‘Cavendish’ and ‘Red’ subgroups. The diploid clones and AAB and ABB hybrids display only two alleles in the diagrammes (Table 4). Horry (1989) and Lebot et al. (1993) mentioned similar results. In contrast, the work of

<table>
<thead>
<tr>
<th>Table 3. Protocols for the visualization of enzymatic activities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIAPHORASE (DIA)</strong></td>
</tr>
<tr>
<td>Tris HCl 1M pH 8.5</td>
</tr>
<tr>
<td>H₂O</td>
</tr>
<tr>
<td>β- NAD</td>
</tr>
<tr>
<td>α-naphtil acetate</td>
</tr>
<tr>
<td>β-naphtil acetate</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
<tr>
<td>Fast Blue RR</td>
</tr>
<tr>
<td>Dissolve the substrate and the stain in the buffer solution and incubate in the dark at ambient temperature for four hours.</td>
</tr>
<tr>
<td><strong>ESTERASE (EST)</strong></td>
</tr>
<tr>
<td>Tris HCl 0.5M pH 7.1</td>
</tr>
<tr>
<td>H₂O</td>
</tr>
<tr>
<td>α-naphtil acetate</td>
</tr>
<tr>
<td>β-naphtil acetate</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
<tr>
<td>Fast Blue RR</td>
</tr>
<tr>
<td>Dissolve the substrates in acetone and then mix the remaining ingredients. Incubate for two hours at ambient temperature.</td>
</tr>
<tr>
<td><strong>MALIC ENZYME (ME)</strong></td>
</tr>
<tr>
<td>Tris malate 0.1M pH 7.2</td>
</tr>
<tr>
<td>MgCl₂ 0.1 M</td>
</tr>
<tr>
<td>β- NAD</td>
</tr>
<tr>
<td>α-naphtil acetate</td>
</tr>
<tr>
<td>β-naphtil acetate</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
<tr>
<td>Fast Blue RR</td>
</tr>
<tr>
<td>Mix and incubate for 45 min at 37°C.</td>
</tr>
<tr>
<td><strong>RUBISCO (RUB)</strong></td>
</tr>
<tr>
<td>Coomassie Blue</td>
</tr>
<tr>
<td>Washing solution:</td>
</tr>
<tr>
<td>M ethanol</td>
</tr>
<tr>
<td>H₂O 100 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Dissolve the Coomassie Blue in 100ml washing solution and filter; mix with the gel and agitate overnight at ambient temperature.</td>
</tr>
<tr>
<td><strong>SHIKIMATE DESHYDROGENASE (SKDH)</strong></td>
</tr>
<tr>
<td>Tris HCl 1M pH 8.5</td>
</tr>
<tr>
<td>H₂O</td>
</tr>
<tr>
<td>Shikimic acid</td>
</tr>
<tr>
<td>β- NAD</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
<tr>
<td>Fast Blue RR</td>
</tr>
<tr>
<td>Mix and incubate for two hours at 37°C.</td>
</tr>
</tbody>
</table>

ABB triploid zones display considerable variability, with observation of five of the six alleles observed (Table 4). The two diploid clones display the same two zones of enzymatic activity as the triploid clones. Malate dehydrogenase (MDH): a single zone of activity was revealed in all the experiments. MDH-1. Our observations confirm the dimeric structure of the enzyme reported by Jarret and Litz (1986b, 1986c). Bhat et al. (1992b) report a high degree of polymorphism in this enzyme, with four zones of enzymatic activity. Three alleles were observed in the AAA clones, including the ‘Cavendish’ and ‘Red’ subgroups. The diploid clones and AAB and ABB hybrids display only two alleles in the diagrammes (Table 4). Horry (1989) and Lebot et al. (1993) mentioned similar results. In contrast, the work of
Table 4. Number of bands per locus detected at different ploidy levels.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Total number of bands</th>
<th>AA</th>
<th>AAA</th>
<th>AAB</th>
<th>ABB</th>
<th>Number of common bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dia-1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dia-2</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dia-3</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Dia-4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Est-1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Est-2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Est-3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Est-4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Est-5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Me-1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Me-2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mdh-1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pgm-1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pgm-2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Pgdh-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pgdh-2</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Skdh-1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Skdh-2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Rub-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prx-1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>53</td>
<td>34</td>
<td>43</td>
<td>32</td>
<td>38</td>
<td>30</td>
</tr>
</tbody>
</table>

Bhat et al. (1992b) showed substantial polymorphism in these groups.

**Diaphorases (DIA):** four zones of activity are shown, with 11 alleles distributed between the different ploidy levels. The most anodic zone, Dia-1, displays a double band for all the clones that can be attributed to a monomeric core structure. Zones Dia-2 and Dia-3 each display four alleles, also corresponding to a monomeric structure. The most cathodic zone, Dia-4, displayed a single band for all the clones tested (Figure 2).

This system displays a high degree of polymorphism in two zones, Dia-2 and Dia-3, for the clones studied (Table 4). This polymorphic, clear and steady system is considered by some authors as a good marker for the identification of cultivars, although correspondence with intraspecific differentiation is not obvious (Giraldo 1996, Maquet et al. 1993, Koenig and Gepts 1989, Kiang and Gorman 1983).

**Malic enzyme (ME):** two zones of activity are shown: Me-1 displays two variants with one or three bands (homo- dimer and heterodimer forms), Me-2 displays a single monomorphic band in all the clones studied (Figure 2). Jarret and Litz (1986b) report the appearance of this band for the genus Musa, with strong activity in starch gels.

**Phosphogluconate dehydrogenase (PGDH):** this enzyme reveals two zones of activity. Pgdh-1 has a single monomeric form for all the clones whereas Pgdh-2 is seen as a dimer, with three bands for the heterozygotic genotype. These results are in agreement with those of Jarret and Litz (1986b), who reported the presence of three bands in the cathodic zone Pgdh-2, suggesting heterozygosity with a single locus.

**Rubisco (RUB):** the RuB-1 zone was very cathodic and monomorphic, with a single band for all the genotypes. Other bands were visible but too attenuated for accurate interpretation.

**Peroxidase (PRX):** at least two zones of enzymatic activity are visible. Only zone Prx-1 can be interpreted as its resolution in acrylamide gel is good. This zone displays a monomorphic model for all the clones (a double band in the different ploidy groups (Figure 2). A second very attenuated zone with low resolution in the mid-region of the gel cannot be interpreted. Bonner et al. (1974), Jarret and Litz (1986c) and Bhat et al. (1992b) have reported several very polymorphic zones of activity in work on other genotypes.

Overall, differences were detected within and between the levels of ploidy. A total of 20 zones (attributed to 20 loci) and 53 bands were identified. An average of 2.65 bands per locus was thus estimated. The average of polymorphic loci was 3.13 bands per locus (Table 4).

The enzymes EST and DIA display the largest number of bands with 14 and 11 respectively, representing 47% of the polymorphism observed in this study. The enzymes PGM, SKDH, PGDH and ME enabled differentiation between the clones derived from M. acuminata (AA and AAA bananas) and hybrid varieties derived from M. acuminata x M. balbisiana (ABB and AAB bananas).

These results will be used in the in extenso study of the Colombian Collection of Musaceae accessions, which totals nearly 130 clones.
The genus Musa originated in Southeast Asia and Vietnam is located within the centre of diversity of cultivated bananas. Several exploration missions have already gathered and evaluated the banana germplasm of Thailand, Malaysia, Indonesia, the Philippines, Papua New Guinea and India, but the banana genetic resources of Vietnam had never been systematically explored and evaluated due to the country’s extended struggle for freedom. In 1993, Prof. Nguyen Dang Khoi, former chairman of Vietnam’s Plant Genetic Resources System and deputy director of Vietnam Agricultural Science Institute submitted to INIBAP a proposal on “Collection, Characterization, Evaluation and Conservation of the Indigenous Musa Germplasm of Vietnam”. INIBAP/IPGRI agreed to fund the proposal in January 1994 and the first prospecting mission was launched in April of that year. The leading workers included Prof. Nguyen Dang Khoi, project coordinator, Vietnam Agricultural Sciences Institute (VASI); Mr. Le Dinh Danh, director of the Phu Ho Fruit Research Centre; Dr. Nguyen Minh Chau, director of the Long Dinh Fruit Research Centre and Dr. Ho Huu Nhi, head of agro-biotechnology department of VASI.

Geography, prospecting missions, indigenous germplasm and field collections

Vietnam occupies the eastern portion of the Indochina peninsula and its coastline straddles the South China Sea. Its western border along Laos is mountainous but on the southern boundaries with Cambodia, the Mekong delta lands fanning out from the Mekong River in South Vietnam and the Red River in North Vietnam. The country extends from 8°30’N latitude in the Gulf of Thailand to 23°22’N latitude bordering China. With Vietnam stretching more than 1,600 kilometres from the mountainous far north to the flat Mekong delta lands in the south, its climate is marked by pronounced regional variations. The northern regions experience cool winters and hot summers while much of central and southern Vietnam enjoy a year-round tropical climate. Southern seasonal variations are wet and dry compared to the hot and cold weather of North Vietnam. The variations in climate and ecosystems promote diversity in the indigenous banana germplasm.

The project was classified into two major activities. The first phase concentrated on the organization and launching of five prospecting missions and the establishment of field collections. These activities were under the leadership of Director Le Dinh Danh. He was assisted by staff members of Phu Ho and Long Dinh Fruit Research Centres. Mr. H. Tézanas du Montcel (banana taxonomist) and Dr. David Jones (banana pathologist), former senior scientists of INIBAP, joined the prospecting mission in North Vietnam. The standard IPGRI/INIBAP format for collecting descriptor information was adopted. Healthy suckers from each collected accession were planted at Phu Ho Fruit Research Centre, site of Vietnam’s National banana field collection. Germplasm materials collected from South Vietnam were established at Long Dinh Fruit Research Centre and duplicated at Phu Ho. Table 1 presents information regarding prospection dates, regions covered and number of accessions collected in the five prospecting missions.

The five prospecting missions visited 30 provinces out of the total 43 provinces in the country. The banana collectors travelled approximately 5,000 kilometres. Figure 1 illustrates the ecological regions covered by the collecting teams. Two wild species, Musa balbisiana Colla and Musa itinerans Cheesman were encountered widely dispersed all over the country. In many locations, these two wild species were found growing side by side. Musa acuminata Colla and Musa coccinea Andrews were observed only in South Vietnam while sparse populations of Ensete glaucum

Le Dinh Danh, Ho Huu Nhi and Ramon V. Valmayor

This work was carried out within the framework of the CORPOICA national plant genetic resources programme, with support from INIBAP and COLCIENCIAS.

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Roxb. were recorded primarily in the cooler northern regions. Some wild species of ornamental value such as Musa laterita Cheesman and Musa coccinea Andrews have been domesticated and are grown in the gardens around temples and pagodas constructed at the fringes of densely forested areas. Pisang Seribu, the thousand-fruited banana formerly known as Musa chiliocarpa Backer and locally called Chuoi Tram Nai, is popular in South Vietnam. The term Chuoi, which means banana, is commonly used as a prefix to the actual varietal name in the same way Chiao is used in China, Klui in Thailand and Pisang in Malaysia and Indonesia. Many wild species of Musa are given the name Chuoi Rung meaning jungle banana. To differentiate the various kinds of wild species, a second or third descriptive term is usually attached such as Chuoi Hot Rung — seeded jungle banana and Chuoi Rung Hoa Sen — wild banana with lotus colored bracts.

The Vietnam prospection yielded many interesting but undescribed accessions belonging to the Musaceae family. A unique accession, which looked like Musa itinerans (stoloniferous plant bearing seedy fruits with shiny, purple-brown male bud), differs from the type species in the manner of bract curling. Instead of rolling upwards as in Musa acuminata, its revolute bracts twist sideways exposing the whitish under surface as the bracts roll (Figure 2). Two other undescribed specimens that arise from stolons are Chuoi Rung Hoa Do and Chuoi Rung Hoa Sen. The former bears an erect inflorescence with orange-red bracts and yellow male flowers. The female flowers at the base are uniseriate and develop into thin, unfilled fruits which are yellow-orange colour even when immature. The fruits are pendant and point downward (Figure 3). Chuoi Rung Hoa Sen is a very similar accession which also produces an erect inflorescence but the rachis bends slightly at maturity. Its uniseriate male flowers are coloured yellow and the fruits are reflexed and point to the ground, but the bract colour is lilac, fruits are normal, green at immature stage and seedy. A very unique wild banana called Chuoi Rung Hoa Soan bears bright yellow bracts with distinctive dried up tips arranged spirally on the markedly imbricate male bud. Perhaps the most highly ornamental accession is the unclassified specimen collected from the grounds of Cuc Phuong Forest Reservation (Figure 4). The plants grow to about 1,5 meters tall and are densely suckering. The inflorescence is upright, the bracts bright orange with yellow slightly imbricate tips. The male flowers are rich yellow with green lobes. Fruits are also yellow, seedless and arranged uniseriately at the basal hands. But the greatest find of the Vietnam banana prospection project is Chuoi Canh, which is neither a Musa nor an Ensete (Figure 5). This highly ornamental Musaceae is awaiting formal description by a trained botanist.

Characterization and conservation

Characterization and conservation constituted the second phase of the Vietnam banana prospection project. Musa germplasm classification was accomplished by Dr Le Dinh Danh at Phu Ho National Banana Field Collection in

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Table 1. Missions and dates, ecological regions and number of accessions collected.

<table>
<thead>
<tr>
<th>Missions</th>
<th>Prospection date</th>
<th>Ecological region</th>
<th>N° of accessions collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Central Vietnam (Northern provinces)</td>
<td>26 2 28</td>
</tr>
<tr>
<td>1</td>
<td>April 6-17, 1994</td>
<td>Central Vietnam (Northern provinces)</td>
<td>26 2 28</td>
</tr>
<tr>
<td>2</td>
<td>September 13-24, 1994</td>
<td>Northern region (Mountainous)</td>
<td>10 4 14</td>
</tr>
<tr>
<td>3</td>
<td>September 25-27, 1994</td>
<td>Red River delta</td>
<td>8 2 10</td>
</tr>
<tr>
<td>4</td>
<td>November 28 to December 4, 1994</td>
<td>North western region</td>
<td>6 4 10</td>
</tr>
<tr>
<td>5</td>
<td>November 25 to January 7, 1995</td>
<td>Mekong River delta and Southern provinces of Central Vietnam</td>
<td>38 7 45</td>
</tr>
<tr>
<td>Sub Total</td>
<td></td>
<td></td>
<td>88 19 107</td>
</tr>
<tr>
<td>N° of accessions in original collection at Phu Ho</td>
<td></td>
<td></td>
<td>44 - 44</td>
</tr>
<tr>
<td>Grand Total</td>
<td></td>
<td></td>
<td>132 19 151</td>
</tr>
</tbody>
</table>

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Figure 2. Chuoi Rung: Close up of bract twisting sideways as it rolls open

Figure 3. Close up of yellow-orange fruits and orange-red bracts of Chuoi Rung Hoa Do

Figure 4. An unnamed and undescribed species of Musa collected from Cuc Phuong Forest Reservation

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North Vietnam. He used the standard IPGRI/INIBAP Musa Descriptors in classifying the Musaceae accessions and adapted the Silayoi and Chomchalo\textsuperscript{a} taxonomic score card in identifying the genome composition of cultivars. Characterization started when the germplasm collection began to flower and fruit. Cytological studies of the entire collection are ongoing and isozyme analysis will be undertaken. Results will finalize the preliminary classification presented in Table 2. The morpho-taxonomic studies at Phu Ho and Long Dinh revealed many duplicates and synonyms. Of the 19 wild species, only 11 were found to be distinct accessions. The remaining eight were considered as duplicates. A similar situation was observed in the characterization of cultivars. Only 64 distinct cultivars were identified out of the total 132 accessions. The balance of 68 entries were found to be duplicates and synonyms and were discarded. Table 2 presents the list of wild and cultivated bananas in Vietnam.

Banana classification is most interesting in Vietnam not only for the many new, unidentified species but also because the taxonomist can observe various stages in the evolutionary transition from wild and seedy diploid Musa balbisiana (BBw) to edible, seedless and pure triploid Musa balbisiana cultivars (BBBc). Vietnamese farmers and scientists recognised three forms of balbisiana bananas. One is the wild and very seedy species that grow in open grasslands and clearings in jungles locally called Ch\textsuperscript{i}oi Hot Rung. Members of the second form are also seedy but the seeds are soft and not so numerous. They are classified as cultivated plants as they are actually planted for various purposes. The male flower bud and the central cylinder are eaten as salad and vegetable. The leaves are used for wrapping local food delicacies and the fruit and pseudostem are fed to hogs. According to folk taxonomy in Vietnam, two types are identified under the second, transitory balbisiana form and they are called Ch\textsuperscript{u}oi Hot (Figure 6) and Ch\textsuperscript{i}oi Hot Qua Lep. They are differentiated primarily on the degree of seediness. The plants belonging to the third form bear edible and seedless fruits. They look very similar to Saba of the Philippines, Pisang Nipah of Malaysia and Pisang Kepok of Indonesia where they are classified as pure balbisiana cultivars (BBBc). Examples are Ch\textsuperscript{u}oi Ngu (Figure 7), Ch\textsuperscript{i}oi Mat (Figure 8) and Ch\textsuperscript{u}oi Sap. Literature on banana classification describes Musa balbisiana as highly uniform and no subspecies are recognised as in the case of Musa acuminata. But in fact, banana taxonomists in Southeast Asia and India are aware of the variability that exists in wild Musa balbisiana. In Vietnam, two kinds are recognised. The more common kind bears ovate male buds, which are markedly imbricated. The entire bracts lift at an upright position approaching 45°. The second kind bears spherical male buds with convolute bracts at the apex. Entire bracts lift but only up to the horizontal position.

Classification of indigenous Musaceae of Vietnam is a dynamic activity. Seven accessions remain unidentified, six at species level and one at genus level. The search for old literature published in Vietnamese, French and Chinese is continuing. The authors will welcome information that will unravel the correct identity of the unknown but very inter-

### Table 2. List of accessions in the national banana field collection at Phu Ho, Vinh Phu, Vietnam*.

<table>
<thead>
<tr>
<th>Category</th>
<th>Accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Wild and ornamental bananas</strong></td>
<td></td>
</tr>
<tr>
<td>Musa, identified</td>
<td></td>
</tr>
<tr>
<td>Chuoi Hot</td>
<td>Musa balbisiana</td>
</tr>
<tr>
<td>Chuoi Tay</td>
<td>M. acuminata</td>
</tr>
<tr>
<td>Chuoi Rung</td>
<td>M. itinerans</td>
</tr>
<tr>
<td>Chuoi Sen</td>
<td>M. coccinea</td>
</tr>
<tr>
<td>?</td>
<td>M. laterita</td>
</tr>
<tr>
<td><strong>II. Musa, unidentified/undescribed</strong></td>
<td></td>
</tr>
<tr>
<td>Chuoi Rung</td>
<td></td>
</tr>
<tr>
<td>Chuoi Rung Hoa Do</td>
<td></td>
</tr>
<tr>
<td>Chuoi Rung Hoa Sen</td>
<td></td>
</tr>
<tr>
<td>Chuoi Rung Hoa Soan</td>
<td></td>
</tr>
<tr>
<td>Chuoi Cau Rung</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
</tr>
<tr>
<td><strong>III. Ensete</strong></td>
<td></td>
</tr>
<tr>
<td>Chuoi Ngu</td>
<td>Ensete glaucum</td>
</tr>
<tr>
<td><strong>IV. Unidentified genus/section</strong></td>
<td></td>
</tr>
<tr>
<td>Chuoi Canh</td>
<td></td>
</tr>
<tr>
<td><strong>B. Semi-wild, semi-cultivated bananas</strong></td>
<td></td>
</tr>
<tr>
<td>Chuoi Hot</td>
<td>Musa balbisiana</td>
</tr>
<tr>
<td>Chuoi Hot Qua Lep</td>
<td>M. balbisiana, seeds hard and viable</td>
</tr>
<tr>
<td><strong>C. Edible, cultivated bananas</strong></td>
<td></td>
</tr>
<tr>
<td>Cultivars AA</td>
<td>10</td>
</tr>
<tr>
<td>Cultivars AAA</td>
<td>18</td>
</tr>
<tr>
<td>Cultivars AAB</td>
<td>9</td>
</tr>
<tr>
<td>Cultivars AB</td>
<td>10</td>
</tr>
<tr>
<td>Cultivars ABB</td>
<td>13</td>
</tr>
<tr>
<td>Cultivars BBB</td>
<td>3</td>
</tr>
<tr>
<td><strong>D. Introduced cultivars and hybrids</strong></td>
<td></td>
</tr>
<tr>
<td>Cultivars</td>
<td>5</td>
</tr>
<tr>
<td>Hybrids</td>
<td>3</td>
</tr>
</tbody>
</table>

* Editor’s note: Ploidy levels and group classification are assigned from morphological characters only.

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**Figure 5.** Close up of bright yellow inflorescence of Chuoi Canh

**Figure 6.** Chuoi Hot is seedy Musa balbisiana. It is grown in backyard gardens for its leaves, edible male bud and as hog feed. Seeds are not so numerous

**Figure 7.** Chuoi Ngu, bearing typical Musa balbisiana male bud and bracts. This is a triploid BBBc
Establishment of embryogenic callus and initiation and regeneration of embryogenic cell suspensions from female and male immature flowers of Musa

Agnès Grapin, Juan-Luis Ortiz, Régis Domergue, Jacqueline Babeau, Sophie Monmarson, Jean-Vincent Escalant, Claude Teisson and François Côte

In Musa, there are two main reasons for developing an efficient cell regeneration process: (i) as a new mass micropropagation technique and (ii) to have a cell regeneration system useful in the development of genetic engineering techniques.

During the last ten years, four methods of somatic embryogenesis in Musa have been published, each one using a different type of explant. The first results were obtained using immature zygotic embryos (Cronauer and Krikorian 1988, Escalant and Teisson 1988, 1989). However, this technique is limited to wild seminiferous genotypes, and is not suitable for cultivated genotypes. Embryogenic cell suspensions were obtained from leaf tissues and rhizomes (Novak et al. 1989), and from highly proliferating meristem cultures (Dhed’a et al., 1991). For these latter two techniques, little quantitative data on the regeneration efficiencies are available. The fourth technique for somatic embryogenesis, using immature male flowers, was initially developed by Ma (1991). Using temporary immersion, an increased level of embryogenesis was obtained by Escalant et al. 1994, while researchers at CIRAD also obtained embryogenic cell suspensions with a high regeneration rate using immature male flowers (Grapin et al. 1996, Côte et al. 1996). This technique was however limited to genotypes having a persistent male bud. Recently, due to the development at CATIE of a process using immature female flowers, this technique has potentially been extended to all types of Musa (Grapin et al. 1997).

A synthesis of the results obtained to date by CIRAD and CATIE on callogenesis and embryogenic cell suspensions initiated from immature flowers is reported here. Full details of the composition of the culture media used and the four stages of the methodology are provided in Grapin et al. (1996) and Côte et al. (1996).

Initial explants

Male flowers
After bunch development, the male buds are collected. The cultured tissues consist of immature male flowers removed from the bud under sterile conditions. Of the hundreds of floral rows present in the bud, only the hands from the rows nearest to the meristem are cultured. These hands are smaller than 3mm and contain 15 to 20 flowers each.
Female flowers

Plants are cut at their base when they are apparently at the transition between the vegetative and the floral stage. After eliminating the leaves, the pseudostems are opened lengthwise to extract the young bud. For the cv. Currare, the buds contain less than 20 differentiated hands and those formed after the sixth or seventh row contain only one flower.

Development of callus

During culture, the development of the explants is relatively similar whether they originate from female or male flowers. The largest hands of flowers (more than 2mm) continue their growth without forming callus tissues. Necrosis occurs in the smallest hands. Yellow nodular callus appears on medium size hands. After the third month in culture, the first callus with proembryos is visible. This embryogenic callus can vary from callus bearing one to a few embryos to white friable callus with multiple proembryos. Plants can be regenerated by transferring somatic embryos removed from an embryogenic callus onto a germination medium.

The percentage of male buds forming embryogenic callus depends on the genotype. At CATIE, the average is 40% and 5% for cv. Grande Naine and cv. Gros Michel respectively. For female flowers, 24% of the cultured buds form embryogenic callus for cv. Currare. Only the hands of the first to the forth row, showing at least three flowers, form embryogenic callus.

Embryogenic cell suspensions

Cell suspensions are established by transferring embryogenic callus to liquid medium. The development and composition of such cell suspensions are relatively similar, whether they originate from female or male flowers, and whatever the genotype used. Cytological studies were carried out on various genotypes to determine their cellular composition and their mode of multiplication.

After several months of subculture, the suspensions contain mainly cell aggregates with a friable structure. These essentially consist of cells with a large nucleus, usually with a single nucleolus. The cytoplasm is rich in soluble proteins, and contains small vacuoles with some protein reserves. Starch reserves are rare or even absent. Internal cell division rich in cytokinin. With a zoom microscope, the first proembryos are visible on the aggregates fifteen days after plating. Cytological studies of somatic embryo ontogenesis demonstrated that a unicellular origin was more than likely. After 45 days, the embryos present an epidermis, a caulinary meristem, a root pole and a provascular system. They have very few reserves, even after a longer period of culture.

The regeneration efficiency quantification was reproduced several times. For the cv. French Sombre, plating 1 ml of packed cells led to the formation of $10^5$ embryos of which 10 to 40% could be converted into plantlets.

For the cv. Grand Naine, plating 1 ml of packed cells led to the formation of $3.7 \times 10^5$ embryos with an average rate of germination of 5%. For the cv. Currare Enano (cell suspension initiated from female flowers), 1 ml of packed cells could result in the formation of $10^6$ to $5 \times 10^6$ embryos.

Conclusion

Immature flowers cultivated in vitro can result in embryogenic callus formation and embryogenic cell suspension initiation for many genotypes of Musa belonging to different groups and different levels of ploidy (Table 1). The development of a methodology for the culture of immature female flowers means that genotypes without male buds, such as AAB False Horn types, can also be included. It is interesting to note that the culture methodology was appropriate for all genotypes studied and modifications were not required. Moreover, this technique of initiating embryogenic cell suspensions has proved to be repeatable in several different laboratories.

Every genotype from which more than 20 to 30 male buds have been cultured...
has produced embryogenic callus followed by cell suspensions and plantlet regeneration. For the genotype Col 49, the culture of only two buds led to the formation of two calli and then two embryogenic cell suspensions.

Some cell suspensions have been subcultured for more than two years, during which time they have maintained their embryogenic capacities. In order to simplify cell suspension management, it is possible to store suspension cultures by cryopreservation using a protocol adapted from Panis et al. (1990).

It has been demonstrated on various genotypes that this embryogenic cell suspension regeneration technique can be very efficient. The technique using immature male flowers has already been successfully used for the development of a genetic transformation method using particle bombardment. It is also being used in an EU- INCO project, one aim of which is the creation of triploid hybrids by somatic fusion. Finally, this regeneration technique is being tested to evaluate its potential as a system for mass propagation. ■

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Propagation and diffusion of improved banana varieties in the Kagera region

P. Rwezaula, C. Hemelings, A. Gallez and S. Sharrock

The most important staple food crop for around 1,000,000 people living in the Kagera region of Tanzania is banana. In recent years yields have declined, mainly as a result of increasing pest and disease attack, declining soil fertility and drought. Severe flooding in some areas, attributed to the El Niño phenomenon, has compounded the effects of poor yields in several parts of the region (Figure 1). Poor banana yields are causing increasing food insecurity and declining health and living standards throughout the region.

Due to their weak financial position, farmers are unable to afford the pesticides and fertilizers necessary for im-

Table 1. Cultivated genotypes with obtained results.

<table>
<thead>
<tr>
<th>Explants</th>
<th>Type</th>
<th>Genotype</th>
<th>Embryogenic callus formation</th>
<th>Subculture and regeneration of embryogenic cell suspensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male flowers</td>
<td>AA</td>
<td>903</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>col 49</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td></td>
<td></td>
<td>SF 265</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>AAA</td>
<td>Grand Nain</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gros Michel</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yangambi</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>AAB</td>
<td>French Sombre</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dominico</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mysore</td>
<td>✓</td>
<td>not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Silk</td>
<td>✓</td>
<td>not tested</td>
</tr>
<tr>
<td></td>
<td>AAAB</td>
<td>PHIA 1</td>
<td>✓</td>
<td>Initiation in progress</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHIA 2</td>
<td>✓</td>
<td>Initiation in progress</td>
</tr>
<tr>
<td>Female flowers</td>
<td>AAB</td>
<td>Currare</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Currare Enano</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

✓: yes

Figure 1. Effects of El Niño

Pp. 181-188 in Proceedings of symposium on tissue culture of horticultural crops. Taipei, Taiwan 8-9
proving the yields of local banana varieties. However, improved banana varieties have been identified which are resistant to many of the pests and diseases affecting the region and which are tolerant of low soil fertility and poor growing conditions.

In an effort to improve the standard of living of farmers in the region, the Kagera Community Development Programme (KCDP), with financial support from the Government of Belgium, has initiated a project to propagate and disseminate improved banana varieties in the region.

The project covers all five districts in the region, thus involving 1,000,000 people and an estimated 180,000 hectares of bananas. It is anticipated that farmers may be prepared to replace up to 15% of their current banana plants with the new varieties, and therefore some 20 million plants may be needed. It is recognised that three major factors will influence farmers' decisions to adopt the new varieties:

- Acceptable flavour
- Confirmed superiority of productivity
- Availability of planting material

**Table 1.** Banana varieties being evaluated in Kagera region

<table>
<thead>
<tr>
<th>Variety</th>
<th>Proposed use</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA-01</td>
<td>M atoke</td>
</tr>
<tr>
<td>FHIA-02</td>
<td>M atoke, dessert</td>
</tr>
<tr>
<td>FHIA-17</td>
<td>M atoke, dessert</td>
</tr>
<tr>
<td>FHIA-23</td>
<td>M atoke, dessert</td>
</tr>
<tr>
<td>I.C. 2</td>
<td>M atoke, dessert</td>
</tr>
<tr>
<td>AA cv. Rose</td>
<td>Brewing, dessert</td>
</tr>
<tr>
<td>FHIA-03</td>
<td>Brewing, dessert</td>
</tr>
<tr>
<td>Karamasenge</td>
<td>Brewing, dessert</td>
</tr>
<tr>
<td>Pisang Berlin</td>
<td>Brewing, dessert</td>
</tr>
<tr>
<td>Pisang Ceylan</td>
<td>Brewing, dessert</td>
</tr>
<tr>
<td>Yangambi Km 5</td>
<td>Brewing, dessert</td>
</tr>
<tr>
<td>SH-3436-9</td>
<td>Dessert</td>
</tr>
<tr>
<td>Cardaba</td>
<td>M atoke, roasting</td>
</tr>
<tr>
<td>Pelipita</td>
<td>M atoke, roasting</td>
</tr>
<tr>
<td>Saba</td>
<td>M atoke, roasting</td>
</tr>
</tbody>
</table>

Note: The proposed uses are tentative for some of the varieties and will be further updated according to the results of the palatability tests.

**Project activities**

Fifteen varieties have so far been selected for testing by the project (Table 1).

Initial planting material is multiplied in vitro at the tissue culture laboratory of the Katholieke Universiteit Leuven (KUL) in Belgium. Rooted plantlets are sent to Tanzania through the Tropical Pesticide Research Institute (TPRI) in Arusha to strictly satisfy the quarantine regulations. Plantlets are then shipped to Kagera where they are weaned and hardened and established in field nurseries for further multiplication.

In the initial stages of the project, a weaning nursery was constructed using local materials — bamboo and hessian. This was used to successfully wean and harden the first batch of 1,000 plantlets. From the total batch, only 80 plants (0.8%) were lost during this stage. A larger, more permanent nursery was later built in order to handle the larger numbers of plants being introduced. This nursery can hold 10,000 plants at any one time and can thus handle 50–60,000 plants per year (Figure 2).

After two months in the nursery, plants are ready to be planted in the field multiplication plots. A number of such plots have been established in two of the five districts during 1997 and the remainder will be established in 1998. At each multiplication site, a demonstration plot is also planted in order to allow local farmers to see the performance of the various new varieties. Multiplication sites are run through collaboration between NGOs, Farm Extension Centres, Institutes and individual farmers (Figures 3, 4).

**Evaluation**

Systems of banana production in Kagera region are variable as a result of varying combinations of environmental conditions, varieties and management techniques. KCDP has therefore signed an agreement with the Agricultural Research Institute (ARI Maruku) to test the new varieties under the diverse environmental conditions of the region.

**Intensive evaluation**

In Bukoba district, three villages have been selected to represent high, medium and low rainfall areas. Farmers who are involved in the evaluation work have been selected to represent rich, medium and poor families, taking into account both male and female headed households. The aim is to test and make available, recommendations for the acceptability of different varieties under different environments and different management systems.

**Extensive evaluation**

In all five districts, less intensive evaluations are carried out involving local NGOs and District Authorities. Project researchers are responsible for monitoring, data collection, analysis and reporting.

**Preliminary results**

All the new varieties evaluated so far, FHIA-01, -02 and -03 and Yangambi Km5, are growing well. They are not affected by pests and diseases and farmers are starting to select the varieties they prefer according to their morphological characteristics. Final evaluation will of course not be possible until the plants have flowered.

**Training**

Four scientists/technicians have been sent to Uganda for training at NARO/IITA on the handling of in vitro plantlets, pot-
Effects of shade

Musa in multistrata systems: Focus on shade

Lyndsey Norgrove

Multistrata systems

A multistrata system comprises two or more crops at least one of which is a woody perennial, that are stored, horizontally mixed and usually harvested at different times. Examples include growing a wide range of food crops, spices and herbs with fruit trees, cacao, palms, or timber trees. Such systems have been used by indigenous peoples in the humid tropics and described widely in the literature. They have been proposed, as a replacement for slash and burn agriculture as it is hypothesised that they provide ecological benefits as well as diversification of farming systems and increases in the farmer’s cash income (Watson 1983). Yet they have not been thoroughly tested. The way components interact needs to be understood both to improve existing systems and to design new ones.

Any biophysical advantage of a mixed system relative to monoculture production systems will depend upon the species’ combination selected, actual and relative strata densities and the limiting factors of the selected environment. However the crux is that the benefits of combination, the processes of ‘complementarity’ and ‘facilitation’ (Box 1) must outweigh the costs of combination, the interspecific competition for space, light, water and nutrients such that long term productivity is higher. Given that the understory crop is subjected to a competitor with an established network of roots for water and nutrient uptake imposing shade that cannot be escaped by upward growth, it bears most of these interspecific competition costs. The response of the lower stratum crop is thus a useful approximation of the cost: benefit ratio in the short-term. Any economic advantage of multistrata systems is also a function of labour costs and the relative values of the upper and understory products. For example, farmers in Bangladesh maintaining Mangifera indica (mango) and Artocarpus heterophyllus (jackfruit) trees in their fields were aware of yield declines in understorey Oryza sativa (rice) and Triticum aestivum (wheat) occurring up to 7m from the trunk. However these losses were acceptable to them because of the valuable fruit and timber supplied by the trees (Hocking et al. 1997).

The response of Musa species to shade

Musa species have traditionally been used as a lower stratum component in multistrata systems of smallholder farmers in the humid tropics (Table 1). Yet there are few studies in the literature about the effects of tree competition and shade upon growth and yield of Musa spp. All studies found on yield responses to shade used Cavendish (AAA) bananas. In the humid tropical lowlands, Murray (1961) measured the impact of two factors: light flux density at three levels (70, 50 and 25%) established with shading cloth plus a 100% no-shade control; and various fertilizer treatments on the yield of Dwarf Cavendish interplanted with cacao seedlings. Vincente-Chandler et al. (1996) compared Dwarf Cavendish yields from plots shaded with Inga inga trees (50% light flux) with those from unshaded plots; all treatments received fertilizer but were not irrigated. Torquebiau and Akyeampong (1994) assessed the impact of shading cloth at three light flux densities (70, 50, 25%) and an unshaded control on ‘fully fertilized and irrigated’Dwarf Cavendish in the tropical uplands. In the subtropics, Israeli et al. (1996) reported the effects of three light flux densities (81, 50, 32%) established with shade cloth on leaf anatomy and chemistry, photosynthesis and yield of Giant Cavendish rafton crop (R1) under irrigation. Eckstein et al. (1997) discussed the effect of temporary, winter shading at 31% PAR from adjacent vertical windbreaks on photosynthesis and yield of Dwarf Cavendish plant crop (PC) and R1.

Effect of shade on phenology

In all studies, the growth cycle was lengthened under lower light flux densities (Figure 1). Eckstein et al. (1997) found reduced leaf emergence rates (LER) in both the PC and R1 under shade and that 1.87 and 3.4 more leaves were produced, respectively. The shading treatment caused a reduction in temperature and within a certain temperature range, LER is positively correlated with ambient temperature (Robinson and Nel 1988). Robinson and Alberts (1987) calculated that in the subtropics...
a 10°C increase in mean monthly maximum temperature between 23 and 32°C was equivalent to an extra 3.6 leaves year\(^{-1}\). However, LER is also greatly reduced by water stress (Kallarackal et al. 1990, Norgrove and Hauser unpubl.). In the tropics, Goenaga et al. (1993) found that the number of days to flowering of AAB cv. maricongo Horn plantain could be predicted \((r^2 = 0.91)\) from the pan-factor irrigation level such that:

\[
days\ to\ flower = 347.3 - 134.8x + 63x^2
\]

where \(x\) = pan factor.

**Effect of shade on yield**

The effects of shade on marketable yields were various (Figure 2). In the subtropics, there was a negative response to any level of shading. Israel et al. (1996) found that R1 yield = 0.21 (% light flux) + 7.61; PC yield was not quoted. Eckstein et al. (1997) found an insignificant reduction in PC bunch mass yet a significant reduction in the R1 with fewer hands per bunch under temporary shading. Photosynthesis rate in the R1 was reduced by 27% with shading. Productivity (bunch weight/time from planting to harvest) was reduced in shaded treatments in both experiments. The prolonged growth cycle could not fully compensate for the reduced photosynthesis rates. In the subtropics, the lengthening of the vegetative stage may delay flower initiation into the coldest months which reduces bunch mass (Robinson and Human 1988).

In contrast, all studies in the tropics showed a positive effect of shade on yield. Murray (1961) reported that bunch mass was greatest in the 50% light flux density treatment. When NPK fertilizer was applied, it was greatest at 75% light. Vincecente-Chandler et al. (1966) obtained bunch masses of 14.2 kg (7.1 hands) under shade trees yet 10 kg (6.3 hands) under full light of which only 4.8 kg (3.6 hands) were deemed marketable. Torquebiau and Akyeampong (1994) found that bunch mass was greatest under 50% shading. In the three experi-
ments quoted, productivity was higher at 70% light flux than in full light (Figure 3). This seems to disagree with the theory that the relationship between total dry matter accumulated and intercepted flux density is approximately linear when light is limiting (Monteith 1977, Russell et al. 1989) thus radiation use efficiency (RUE: biomass accumulated/total solar radiation intercepted) is constant for a given species. The above data suggest that the RUE is not constant but increases under low light flux densities (Figure 4). To explain this discrepancy, the following hypotheses are suggested here and expanded below:

- At full light intensities, light flux densities exceed the PAR saturation density of Musa leaves
- Radiation use efficiency (RUE) changes as light quality changes
- Resource allocation within the plant changes
- Other factors may be limiting and the shade treatment was interacting with these factors
- Light level affects pest and disease dynamics to the benefit of the crop
- Light level affects weed: crop relations to the benefit of the crop.

At full light intensities, light flux densities exceed the light saturation density of Musa leaves

Work by Gietema-Groenendijk (unpublished M.Sc. thesis, 1970, quoted in Samson 1980) showed that banana leaves had a light saturation intensity of 210 W m$^{-2}$ at a leaf temperature of 32°C. This is approximately 20% of the light flux density on a cloudless day in the tropics (Samson 1980). In addition, at very high light intensities, both photorespiration (Eckstein et al. 1997) and necrotic scorching of peel (Wade et al. 1993) reduce marketable yield.

Radiation use efficiency (RUE) changes as light quality changes

A tree canopy not only reduces the light flux density to the lower storey, it changes its quality. The absorptive, transmissive and scattering properties of the canopy (Gay et al. 1971) increase the proportion of far-red, near infrared and diffuse radiation (Boardman 1977). Crops may increase their RUEs as the percentage of diffuse radiation increases (Sinclair et al. 1992). No references to Musa spp. were found.

Resource allocation within the plant changes

Shaded plants usually have thinner leaves, higher specific leaf area, larger chloroplasts and higher chlorophyll concentrations (Boardman 1977). This has been shown both in experiments comparing shaded and lit leaves on the same plant (e.g. rainforest trees, Hernandez et al. 1995) and in shade-cloth experiments (e.g. cacao, Galyuon et al. 1996a and 1996b). Musa spp. also exhibit morphological and physiological changes if grown in the shade. Balasimha (1989) showed that a Musa sp. grown in the understorey of arecanut palms adapted to...
lower light levels by producing thinner leaves with higher chlorophyll content, specific leaf area and photosynthetic efficiency. Murray (1961) measured the leaf area of the third leaf six months after planting. Leaf area was greatest in the 50% light treatment and within shade treatments, it was positively correlated with bunch weight. Foliar analysis showed a negative correlation between % light and ash, N, P, and K foliar concentrations, suggesting higher chlorophyll levels in the leaves from shaded treatments. Resource allocation is adjusted in shaded plants, increasing both photosynthetic area and chlorophyll contents to compensate for reduced light fluxes.

The different temperature regimes imposed by a shading treatment may affect resource allocation between different plant organs such that the harvest index (marketable yield/total biomass *100%) is increased. Turner and Lahav (1983) investigated the impact of various controlled temperature regimes ranging from day/night couples of 17/10°C to 37/30°C upon resource allocation in Giant Cavendish (AAA) in growth chambers. They found that temperature regime strongly affected resource allocation. They calculated optimum temperatures for maximum leaf emergence rate (31.6°C), leaf area (31.1°C), and dry wt increment (21.2°C). The largest corms were obtained at 21°C and pseudostems at 25°C daytime temperatures. Corm mass is positively correlated with bunch size (Turner and Lahav 1983). Stolar (1962) found that bunch mass was greatest when pseudostem temperature was between 21-24°C. As shaded plots in the tropics have lower temperatures, the harvest index may increase under shade.

Other factors may be limiting and the shade treatment was interacting with these factors

Light levels may interact with nutrient and water status where these are limiting. Experiments with cacao have shown an interaction between nutrition and light with greater yield responses to fertilizer under full light (Cunningham and Arnold 1962, Ahenkorah et al. 1987). Murray (1961) found that bunch mass was always greater in fertilized treatments, yet there was a greater positive response to fertilizer at higher light flux densities. In the work by Vincente-Chandler et al. (1966), the shade treatment imposed was an upperstorey of Inga inga (L.). This is a Leguminosae and it is possible that nitrogen fixation was occurring and this contributed to the higher yields under trees as nutrients were more limiting than light.

Musa spp. require large amounts of water for maximum production: a minimum of 25 mm week<sup>-1</sup> (Purseglove 1972). In tropical areas with pronounced dry seasons, water deficit limits growth. Many studies have shown the beneficial effects of frequent irrigation upon yield (Goenaga et al. 1993; Hegde and Srinivas 1989). Shading interacts with water deficit by reducing plant demand and reducing evaporation from the soil surface. Eckstein et al. (1997) found reductions...
in transpiration rates and stomatal conductance of 38% and 40% respectively. In the tropics, a Dwarf Cavendish plant uses 25 litres of water on a clear day, 18 litres on a cloudy day and 9.5 litres on an overcast day (Champion 1963). In non-irrigated systems, shading may offset water deficit and this gain may be greater than any loss caused by light deficiency.

**Indirect effects of shade: impact on foliar diseases**

Shade may reduce the degree of damage from *Mycosphaerella fijiensis* (Morelet) leaf spot fungus (black Sigatoka), a major constraint for Musa production in tropical humid areas. Germination of the infective agents, conidia and ascospores, requires high humidity. Early growth is influenced by temperature with maximum growth of conidia and ascospore germ tubes at 25-28°C (Stover 1986, jcom et al. 1991, Porras and Pérez 1997). Shading reduces air temperature below the optimum temperature therefore infection may be reduced. Vincente-Chandler et al. (1996) found that 49.1% of banana leaves from plants in unshaded plots were severely damaged compared to 25% in shaded plots.

**Indirect effects of shade: impact on weeds**

The growth of many common arable weeds, particularly C4 grasses, is greatly reduced by shading. In the humid tropics, Ng et al. (1997) showed that the biomasses of various grass species in rubber plantations with light levels of 65% PAR of ambient light (G65) were reduced compared to those at 90% PAR (G90). In particular, % reduction (G65 - G90/G90 *100) was greatest in the species that produced the most biomass at 90% PAR:

(1) G65 - G90/G90 *100% = 25.1

In contrast, a comparison of biomasses of legumes showed a linear reduction \( r^2 = 0.73, p>0.001 \) in growth from 90% (L90) to 65% PAR (L65):

(2) L65 = 0.4 L90 + 41.8 (calculated from Ng et al. 1997)

Shade reduced weed biomass and acted as a selection tool, having the most severe impact upon high biomass grasses, which would be likely to negatively affect the growth of any understorey crop. Similar results were found by Shetty et al. (1982): plant height, leaf area index and tuber production of *Cyperus rotundus*, *the most noxious perennial weed in the semi-arid tropics* were positively correlated with light flux density.

**Conclusions**

There have been few studies on yield responses of Musa to the shade and competition from upper canopy trees. Analyses of these studies suggest positive yield and productivity responses to some degree of shade. Radiation use efficiency was not constant but increased at lower light flux densities. Increases of RUE under shade was also reported by Manrique et al. (1991), working on *Solanum tuberosum* (L.) (potato).

All but one study quoted used shade cloth to establish the light treatments and this has a number of drawbacks as a tree-mimic. Shade cloth, unlike trees, does not change the light quality reaching the understory. The percentage of PAR passing through is proportional to the percentage of total light passing through (Eckstein et al. 1997) whereas trees use PAR so that light reaching the canopy will have relatively less. Second, shade cloth may protect against wind damage (Murray 1961) and affect microclimate in a way unlike the action of trees. Third, such experiments cannot measure interactions between light levels and water/nutrient competition which would be present in a real multistrata system.

Clearly more research is needed. In particular, a comparison of the responses of different cultivars is required: plantains (AAB) and cooking bananas (ABB) should be tested as these are staples in many parts of the tropics. As severity of foliar diseases, weed biomass, and soil and water status interact with shade treatments, a multidisciplinary approach is required to design optimum systems. Two such experiments have been started near the International Institute of Tropical Agriculture Humid Forest Station near Mbalmayo, Cameroon. French plantain (AAB) cv. 'Essong' was planted as an understory crop under various crop management regimes in young and old timber plantations and various timber stand density treatments were imposed. The aim of the trial is to quantify the impact of soil and water competition and shading by the trees on plantain growth and yield. Effects of the treatments upon weed community composition and damage caused by *M. fijiensis*, *Radopholus similis* (burrowing nematode) and *Cosmopolites sordidus* (banana borer weevil) are also assessed. These data will then be used to design multistrata systems with optimum upperstorey densities for both acceptable crop yields and maintenance of the resource base in the long term.

**References**


The author is from King's College, London and is currently at the International Institute of Tropical Agriculture (IITA), Humid Forest Station, B.P. 2008 (Messa), Yaoundé, Cameroon.
Selection of plant type for banana (Musa AAA) nematode sampling

M. Araya and A. Cheves


To suppress the damage, management decisions, which involve cultural, biological, or chemical manipulations, alone or in combination, are available (Robinson 1996, Gowen 1995, Gowen and Quénéhervé 1990). However, the non-fumigant nematicide alternative is the most feasible and widely used.

The decision or recommendation to apply a nematicide is based on the R. similis population density estimated from root samples taken from the base of recently flowered plants (within eight days of flower emergence). When the population density is higher than 10,000 nematodes/100g of roots, the use of nematicides is essential for high yields of export quality fruit. Nematicides are applied to the follower or succeeding suckers. This supposes a high correlation between the R. similis population densities of the flowered plants and their respective follower suckers.

Sampling flowered plants has the advantage of standardizing the plant phenological stage, even when in the field the plants may have different ages. In contrast, it has the disadvantage of having to relate the R. similis population from the flowered plant to that of the succeeding suckers, which could result in inaccurate estimates of the actual population density. Any bias or deviation from the real nematode population will affect the agroecosystem. Overestimates can lead to recommendations to apply nematicides, with the subsequent economical and ecological cost, whereas underestimates can result in insufficient chemical treatment, causing yield losses.

The objectives of this study were to determine which type of plant and which sampling position at the plant base, offers the most reliable sampling site for estimating nematode populations, in order to make decisions on the need to apply nematicides to succeeding suckers.

Materials and methods

General procedure

Seven trials were sequentially developed and assessed as hypotheses in nematode population densities were formulated. In five of the trials, the nematode populations from the flowered plants were compared with the populations in their corresponding follower suckers and/or between both plants and the interspace between them. After selection of the plant type, two trials were set up to determine the best sampling site position at the plant base.

All trials were carried out in long-term ratoon commercial plantations cultivated with ‘Valery’ and ‘Grande Naine’ clones. The plant density varied from 1,700 to 1,850 plants/ha. Bunching plants were supported by tying them to adjacent plants with double polypropylene twine. Normal cultural practices (fertilization, control of weeds and nematodes, and aerial spraying of fungicides to control black Sigatoka) were used. Nematicides were not applied to the areas studied for at least six months prior to sampling. Desuckering was carried out every eight weeks throughout the year, such that any mat comprised a bearing mother plant, a large daughter sucker and a small grand-daughter.

As usual for the area, the total water requirement was supplied by rainfall. The average rainfall (1996) varied between farms from 2,554 to 3,744 mm evenly distributed throughout the year. April and March were the driest months with 93 and 103 mm, respectively. Elaborate drainage structures were provided in each field to disperse excess rainfall and prevent waterlogging. Mean daily maximum/minimum temperatures were 28.4/21.2°C.

The numbers of samples collected per trial varied with the farm. Randomly selected, recently flowered plants (within eight days of flower emergence), called from now on mother plants, were individually sampled. The follower sucker from each mother plant was also sampled at the same time. In trials where the comparison included the interspace between both plants, a sample was also taken from the space between the mother plant and its succeeding sucker. A hole of 13 cm long, 13 cm wide and 30 cm deep was dug with a shovel of rectangular blade (13 cm wide x 50 cm long) at each plant base. Roots from each hole were collected individually, placed in labelled plastic bags, transported to the laboratory, and processed within three days. Nematodes were extracted from 25g root samples by maceration in an electric blender (Taylor and Loegering 1953) for 10 sec at low and 10 sec at high speed. The resulting mixture was washed from the blender through 0.5/0.150/0.038 mm (35/100/400-mesh) sieves. The residue on the 35 and 100 mesh sieves was discarded and that on the 400-mesh sieve washed off into a 250-ml beaker and the nematodes counted as described in Araya et al. (1995).

Population densities of all plant-parasitic nematodes present were determined, but only data on R. similis are presented in detail. All counts were made with an Olympus BH microscope at 4x magnification and values converted to numbers of nematodes per 100g of roots. The evaluations were made as follows.

Trial 1: Comparison between flowered plants and their corresponding follower sucker

Two trials were conducted with ‘Valery’ and one with ‘Grande Naine’. One of the ‘Valery’ trials was located on Productora Tropical farm in a clay loam and the other was located on San Pablo farm in a sandy clay loam. The evaluation of ‘Grande Naine’ was done on Rebusca farm in a sandy loam. Full details of the soil conditions at the trial sites are given in Table 1. In the three trials, the follower sucker height (cm) was measured at sampling time.

Data was analysed independently for each trial. The population density of each nematode genera was compared and correlated with the level in the follower sucker. Follower suckers were grouped into three or four groups (depending on the number of observations in each group) by their height, and the R. similis and Helicotylenchus spp. populations compared and correlated with the levels in the mother plants. In ‘Grande Naine’ the comparison of the Helicotylenchus spp. population with the grouping of heights of follower suckers was not made because it was detected rarely and when present it was in low densities. The succeeding sucker...
height was correlated with the R. similis population density.

**Trial 2: Comparison between flowered plants, their respective follower suckers and the interspace between both plants**

Two trials were set up in mixed plantation areas of ‘Valery’ and ‘Grande Naine’ clones. One trial was conducted on the San Pablo farm in a clay loam and the other on the Productora Tropical farm in a sandy clay loam (Table 1). Data was subjected to ANOVA and mean separation using the Waller-Duncan test. The correlation of each genera population between the follower suckers with the mother plants and follower suckers with the interspace between both plants was determined.

**Trial 3: Sampling position at base of plant**

Two trials were carried out, one on the Productora Tropical farm (‘Valery’ clone) in a clay loam and on the Rebusca farm (‘Grande Naine’ clone) in a sandy loam (Table 1). Forty follower suckers from recently flowered plants (within eight days of flower emergence) were randomly selected for root nematode sampling at each farm. Samples were taken at the sucker base as follows: in front of the sucker, at the right and at the left side of the sucker. During sampling, the orientation position of the inflorescence of the flowered plant with respect to the follower sucker (left, right, front) was registered. For each orientation, the nematode data at that position was transferred and included as an additional treatment in the statistical analysis. Root weight and nematode data from four treatments and 40 replicates were subjected to ANOVA and mean separation using the Waller-Duncan test.

**Results**

**Trial 1: Comparison between flowered plants and their corresponding follower sucker**

The ‘Valery’ and ‘Grande Naine’ follower suckers contained more R. similis than their respective flowered plants in the Productora Tropical (P = 0.03), San Pablo (P = 0.001) and the Rebusca (P = 0.04) farms (Table 2). At the three farms, more than 60% of the succeeding suckers contained a higher R. similis population than the mother plants. The correlation coefficients (r = 0.05, r = 0.006, and r = 0.103, respectively) indicated that there was no linear relationship between mother plants R. similis population densities with the levels in their corresponding follower suckers.

A higher (P = 0.0009) number of Helicotylenchus spp. was found in the ‘Valery’ mother plants than in the follower suckers in the Productora Tropical farm, whereas no difference was detected in the San Pablo (P = 0.91) and the Rebusca (P = 0.67) farms (Table 2). The r values do not support any linear relationship between the populations from both plants.

When they were present, no differences in Meloidogyne spp. and Pratylenchus spp. population densities were found between mother plants and their respective follower suckers in Productora Tropical, San Pablo or Rebusca farm. The r values do not explain the variation in nematode population between mother plants and follower suckers (Table 2).

The comparisons by the height of the follower suckers, revealed that the suckers with a height of 101-150 cm (P = 0.008), and with a height of 151-200 cm (P = 0.002), contained more R. similis than the flowered plants in Productora and San Pablo farms, respectively (Table 3). In the Rebusca farm, suckers with a height of 126-175 cm showed more R. similis than the mother plants, but the difference did not reach a significant level. In the follower suckers the R. similis population densities were independent of sucker height (data not showed). This means that either low or high followers can support low or high R. similis densities and viceversa.

**Trial 2: Comparison between flowered plants, their respective follower suckers and the interspace between both plants**

A higher content of R. similis was found in the succeeding suckers in San Pablo (P = 0.004) and Productora Tropical (P = 0.02) farms. The difference was caused by the mother plants. Although the follower suckers had more R. similis than the interspace between both plants, the populations were not large enough to attain statistical significance (Table 4). At both farms more than 57% and 77% of the succeeding suckers had more R. similis than the interspace between both plants and mother plants, respectively.

<table>
<thead>
<tr>
<th>Table 1. Soil conditions at trial sites.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soil composition</strong></td>
</tr>
<tr>
<td>Trial 1 Productora Tropical</td>
</tr>
<tr>
<td>Trial 1 San Pablo</td>
</tr>
<tr>
<td>Trial 2 Rebusca</td>
</tr>
<tr>
<td>Trial 2 San Pablo</td>
</tr>
<tr>
<td>Trial 2 Productora Tropical</td>
</tr>
<tr>
<td>Trial 3 Productora Tropical</td>
</tr>
<tr>
<td>Trial 3 Rebusca</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Comparison and correlation between Radopholus similis, Helicotylenchus spp., Meloidogyne spp., and Pratylenchus spp. populations extracted from banana (Musa AAA) roots in flowered plants with the densities in their respective follower suckers. Nematodes expressed by 100g of roots.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nematode</strong></td>
</tr>
<tr>
<td><em>Valery</em> clone, Productora Tropical farm (103 observations)</td>
</tr>
<tr>
<td>R. similis</td>
</tr>
<tr>
<td>Helicotylenchus spp.</td>
</tr>
<tr>
<td>Meloidogyne spp.</td>
</tr>
<tr>
<td>Pratylenchus spp.</td>
</tr>
<tr>
<td><em>Valery</em> clone, San Pablo farm (105 observations)</td>
</tr>
<tr>
<td>R. similis</td>
</tr>
<tr>
<td>Helicotylenchus spp.</td>
</tr>
<tr>
<td>Meloidogyne spp.</td>
</tr>
<tr>
<td>Pratylenchus spp.</td>
</tr>
<tr>
<td><em>Grande Naine</em> clone, Rebusca farm (115 observations)</td>
</tr>
<tr>
<td>R. similis</td>
</tr>
<tr>
<td>Helicotylenchus spp.</td>
</tr>
<tr>
<td>Meloidogyne spp.</td>
</tr>
</tbody>
</table>

ES = standard error. Values with the same letter are not significantly different according to paired T test.
Table 3. Comparison and correlation between R. similis populations extracted from banana (Musa AAA) roots in flowered plants with the densities in their respective follower suckers according to their height. Nematodes expressed by 100g of roots.

<table>
<thead>
<tr>
<th>Follower sucker height (cm)</th>
<th>Mean ± ES of flowered plants</th>
<th>Mean ± ES of follower suckers</th>
<th>Correlation (r)</th>
<th>Number of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Valery' clone, Productora Tropical farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 101</td>
<td>29,653a ± 7,087</td>
<td>30,507a ± 4,391</td>
<td>0.22</td>
<td>15</td>
</tr>
<tr>
<td>101-150</td>
<td>25,414b ± 2,841</td>
<td>33,014a ± 3,049</td>
<td>0.10</td>
<td>44</td>
</tr>
<tr>
<td>151-200</td>
<td>26,763a ± 3,390</td>
<td>33,000a ± 4,261</td>
<td>0.01</td>
<td>38</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>27,600a ± 6,427</td>
<td>21,533a ± 8,223</td>
<td>0.44</td>
<td>6</td>
</tr>
<tr>
<td>'Valery' clone, San Pablo farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 151</td>
<td>14,326b ± 2,117</td>
<td>22,685a ± 3,033</td>
<td>0.0001</td>
<td>27</td>
</tr>
<tr>
<td>151-200</td>
<td>19,971b ± 2,266</td>
<td>30,909a ± 2,684</td>
<td>0.006</td>
<td>56</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>24,155a ± 3,467</td>
<td>23,873a ± 4,318</td>
<td>0.007</td>
<td>22</td>
</tr>
<tr>
<td>'Grande Naine' clone, Rebusca farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 125</td>
<td>12,585a ± 2,670</td>
<td>18,466a ± 6,057</td>
<td>0.102</td>
<td>26</td>
</tr>
<tr>
<td>126-175</td>
<td>16,210a ± 3,306</td>
<td>26,341a ± 4,895</td>
<td>0.119</td>
<td>50</td>
</tr>
<tr>
<td>&gt; 175</td>
<td>13,241a ± 4,026</td>
<td>16,679a ± 5,243</td>
<td>- 0.0008</td>
<td>29</td>
</tr>
</tbody>
</table>

ES = standard error. Values with the same letter are not significantly different according to paired T test.

Figure 1. Root weight (g) contained in 5.070 cm³ of soil in the different sampling sites.

Table 4. Comparison of nematode population densities at three sampling points; flowered plants, their respective follower suckers and the interspace between both plants.

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Mean ± ES flowered plants</th>
<th>Mean ± ES follower suckers</th>
<th>Mean ± ES interspace between both plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Pablo farm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. similis</td>
<td>11,546b ± 1,752</td>
<td>26,329a ± 4,169</td>
<td>20,663a ± 2,974</td>
</tr>
<tr>
<td>Helicotylenchus spp.</td>
<td>4,080 ± 782</td>
<td>3,894 ± 756</td>
<td>2,596 ± 679</td>
</tr>
<tr>
<td>Meloidogyne spp.</td>
<td>573 ± 181</td>
<td>347 ± 191</td>
<td>507 ± 151</td>
</tr>
<tr>
<td>Pratylenchus spp.</td>
<td>560 ± 199</td>
<td>378 ± 144</td>
<td>253 ± 179</td>
</tr>
<tr>
<td>Productora Tropical farm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. similis</td>
<td>12,386b ± 2,300</td>
<td>23,900a ± 3,666</td>
<td>21,267a ± 2,985</td>
</tr>
<tr>
<td>Helicotylenchus spp.</td>
<td>4,870 ± 628</td>
<td>3,313 ± 440</td>
<td>4,360 ± 562</td>
</tr>
<tr>
<td>Meloidogyne spp.</td>
<td>107 ± 45</td>
<td>107 ± 54</td>
<td>40 ± 29</td>
</tr>
<tr>
<td>Pratylenchus spp.</td>
<td>200 ± 57</td>
<td>440 ± 98</td>
<td>226 ± 66</td>
</tr>
</tbody>
</table>

Data are means of 30 replicates. Values with the same letter in the row are not significantly different according to Waller-Duncan test.

and Productora, respectively. The correlation coefficients were very low, mainly < 0.4 (data no showed).

Trial 3: Sampling position at base of plant

No difference was detected in root weight (P = 0.16; Figure 1) or between R. similis (P = 0.10), Helicotylenchus spp. (P = 0.74), Meloidogyne spp. (P = 0.80), and Pratylenchus spp. (P = 0.24) population densities as a result of the different sampling positions in the ‘Valery’ clone (Figure 2). The same results were found with the ‘Grande Naine’ clone (data no showed).

Discussion

Higher populations of R. similis were found in the follower suckers than in the flowered plants or the interspace between both plants. The height of follower sucker groupings showed that ‘Valery’ suckers with a height of 100-150 cm (Productora) and with a height of 151-200 cm (San Pablo) supported more R. similis than the mother plants. When suckers were > 200 cm in height, their nematode populations were lower than the flowered plants. In ‘Grande Naine’ (Rebusca), although there was no statistical difference, the suckers between 126-175 cm in height had greater R. similis densities. The smaller shoots contain more nematodes, probably due to their higher root nutrient quality, which makes them more attractive to nematodes and favours nematode reproduction. As plants get older, their root systems decay, changing the root growth indices from increasing rates to decreasing rates. In Uganda high percentages of roots were dead or very necrotic in plants nearing maturity (Speijer et al. 1994). Furthermore, root decay is influenced by plant stage with a higher percentage of dead and rotten roots found in older plants (Speijer and Gold 1996).

Follower sucker infection could occur as a result of nematode movement from the mother plant or through the soil. Suckers emerge from the corm of the mother plant and their roots interweave with the roots of the mother and adjacent plants. Long-term monoculture favours nematode distribution and detection in banana plantations. Radopholus similis is a migratory endoparasite, which allows it to explore the root system more actively and it is found in both functional and non-functional banana roots (Speijer and Gold 1996, Araya and Centeno 1995).
In general Helicotylenchus spp. were found in higher densities in the mother plants. This could be related with the feeding habits of semi-endoparasite (Yeates et al. 1993) or ectoparasite, which feed on subsurface tissue (Droppkin 1989). Banana roots are produced continuously until flowering and during this time there may be more substrate available for nematodes with this feeding habit.

Meloidogyne spp. and Pratylenchus spp. were detected rarely and in low densities which agrees with observations from Araya et al. (1995). R. similis suppress both nematodes by competition for the feeding sites (Davide 1996, Santor and Davide 1992, Quénéhervé 1989). Additionally, Pratylenchus spp. are more common in soil with cool temperatures than in warm soils (Rodríguez 1990).

In local conditions, R. similis is a potential threat to commercial cultivation of banana for export production and there is therefore a need to monitor nematode populations. The plant type normally sampled (recently flowered plants) did not give reliable R. similis population estimates for efficient nematode management in the follower suckers. There was no general relationship between the follower sucker R. similis densities and mother plant or interspace between both plants counts, that would serve as a quantitative factor for converting mother or interspace counts to equivalent suckers counts. It is therefore suggested that follower suckers, which are less than 200 cm in height, coming from recently flowered plants (within eight days of flower emergence) should be sampled to give a more reliable nematode population estimate.

There was no difference in root weight, and R. similis, Helicotylenchus spp., Meloidogyne spp. and Pratylenchus spp. population densities among the different sampling positions. Therefore it is advisable to take the root samples from the front of the sucker in order to reduce the possibility of extracting roots from the mother plant and small shoot.

References


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Erratum

INFOMUSA’s editorial office would like to apologise for the error made on p. 3 of the last issue of INFOMUSA (Vol. 6, No. 2) and on the Musa pest fact sheet No. 2 “The root lesion nematodes of banana: Pratylenchus coffeae and Pratylenchus goodeyi”. The two photographs were inverted by mistake.

The photograph in INFOMUSA is in fact of a Pratylenchus goodeyi nematode, while the photograph on the fact sheet (p. 3) portrays a male and female of the Radopholus similis nematode.

R. similis

P. goodeyi
Black Sigatoka disease control in banana and plantain plantations in Cuba

Luis Pérez Vicente

Bananas (Musa spp. AAA) and plantains (Musa spp. AAB) are important staple food crops in Cuba and are grown on around 46,000 ha, of which bananas occupy more than 2/3 of the total. Cooking bananas (Musa spp. ABB) occupy another 60,000 ha, and have been progressively replacing the AAB plantains, because of the low yields and susceptibility to diseases (mainly black Sigatoka) of these latter cultivars.

Black Sigatoka disease (BS) caused by Mycosphaerella fijiensis is the most important and destructive disease present in banana and plantain plantations in Cuba. First reported in 1991 in the central east part of the country (Vidal 1992), BS is now present in practically all banana and plantain production areas. The disease causes leaf spots that coalesce in necrotic patches of dead leaf tissue causing a drastic reduction of the active photosynthetic leaf area and a consequently reduction of bunch weight and yields. After flowering, untreated plants can lose all their leaves and, as a consequence, bunches do not reach the ground level mechanically reduces the available inoculum.

BS disease epidemiology is dependent on abiotic and biotic factors. Temperature and rainfall patterns (basically the number of hours that a leaf surface remains wet) and inoculum availability are key factors in the speed of development of the disease (Pérez and Mauri 1992, Pérez et al. 1993a, Porras and Pérez 1996). In periods when minimum temperatures are below 20°C and there is little dew deposition or rainfall, disease development is strongly depressed and the intervals between fungicide treatments can be prolonged, and even exceptionally stopped. The disease is spread when windborne ascospore inoculum is produced on heavily diseased leaves inside the plantation or in neighbouring untreated fields planted with susceptible cultivars. The amount of available inoculum has a strong influence on the speed of disease evolution. Heavy disease inoculum causes a shortening of the symptom development time and counteracts the effect that unfavorable conditions can have on disease development (Porras and Pérez 1996). The shortening of the symptom development time results in the rapid development of necrotic patches on the leaves and leads to the formation of high quantities of pseudothecia and ascospores. For this reason, sanitation and reduction of inoculum should be considered the first step in any programme of BS disease management, with treatments based on warnings determined by weather and biological assessments.

During the first two years after the disease was reported, an average of 24 to 27 treatments per year were performed to control the disease in most of the banana plantations, using triazole, morpholines, benomyl + carbamates and carbamate fungicides in oil-water emulsions (Pérez et al. 1993b). The cost of disease control represents some 15% of the total investment in the plantations (excluding those costs related to new irrigation systems).

Integrated management of black Sigatoka disease

Since 1992 to the present, a disease control programme based on an integrated approach (Pérez 1993, Pérez et al. 1995) has been implemented in the main plantations of the country. The integrated management programme is based on:

Generalization of the control measures

Disease management practices must be implemented in all the Musa plantations in a given area. Thus land planted with susceptible cultivars which do not receive fungicide treatments, have being replanted with resistant cultivars. Some ABB cooking banana cultivars, as well as new tetraploids (from FHIA in Honduras), have a good level of resistance to BS disease and are being progressively released to replant large and small untreated private and state plantations. At the same time, plants in abandoned fields have been removed so as not to provide a source of inoculum during the rainy season.

Agronomic practices

Agronomic practices play an important role in the physiological state of the plants as well as on the microclimatic environment inside the plantations, which can led to the creation of favourable conditions for disease development. Among the most important are:

- **Plant density.** Under the same soil conditions and level of disease control, BS development has been shown to be more intense in fields with 2000 plants/ha than in those with 1850 plants/ha. Differences up to two more healthy leaves per plant have been observed in the less dense plantations.

- **Nutrition.** A proper N and K balance is required to achieve efficient BS control. In areas were the K and N are limiting, BS development is more intense due to a low rate of leaf emergence and a lower resistance to the disease.

- **Irrigation practices.** Rainfall in Cuba is a limiting factor for growing bananas and plantains. Irrigation is necessary to obtain high yields during at least six months of the year. Water supply is important to maintain rates of leaf emergence greater than 1-1.2 leaves every 10 days. Lower rates of leaf emergence lead to higher levels of disease in the plantations. At the same time, as wet leaves play an important role in disease development, under canopy irrigation systems are used wherever possible. Plantations with overhead irrigation systems usually have higher disease indices than those with under canopy irrigation.

- **Sanitation.** The systematic (every 7-10 days) pruning of leaves or parts of leaves with mature lesions reduces the period in which these leaves are producing inoculum and has an important impact on the quantity of ascospores that can potentially reach newly emerging leaves. The pruned leaves are deposited on the soil surface and are readily decomposed. On average, a reduction of 6-8 weeks (Figure 1) of the total period of ascospore production can be obtained (Pérez 1996, Pérez et al. 1993a). In addition, the position of the leaves at ground level mechanically reduces the available inoculum.

Use of resistant cultivars

The use of resistant cultivars is the most economical and ecologically safe way to control black Sigatoka disease. In Cuba, several hybrids (FHIA-01, FHIA-02,
Table 1. Reaction of a group of FHIA clones to black Sigatoka disease in plots without protection with fungicides (2).

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Genome</th>
<th>Period (days) of incubation</th>
<th>Period (days) of transition</th>
<th>Active leaves at harvest</th>
<th>Spots</th>
<th>Weight of bunches (kg)</th>
<th>Yields (ton/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA 01</td>
<td>AAAB</td>
<td>43.5b</td>
<td>28.2a</td>
<td>76.4</td>
<td>9</td>
<td>17.3</td>
<td>15.9</td>
</tr>
<tr>
<td>FHIA 02</td>
<td>AAAA</td>
<td>46.9b</td>
<td>31.0a</td>
<td>8</td>
<td>10</td>
<td>13.3</td>
<td>34.8</td>
</tr>
<tr>
<td>FHIA 03</td>
<td>AABB</td>
<td>60.4a</td>
<td>24.1b</td>
<td>10</td>
<td>8</td>
<td>15.5</td>
<td>31.6</td>
</tr>
<tr>
<td>FHIA 18</td>
<td>AAAB</td>
<td>60.4ab</td>
<td>28.7a</td>
<td>12</td>
<td>9</td>
<td>14.3</td>
<td>35.0</td>
</tr>
<tr>
<td>SH 3436</td>
<td>AAAA</td>
<td>52.8c</td>
<td>28.0a</td>
<td>10</td>
<td>7</td>
<td>12.7</td>
<td>9.5</td>
</tr>
<tr>
<td>G. ENANO</td>
<td>AAA</td>
<td>27.9c</td>
<td>16.9c</td>
<td>1</td>
<td>0</td>
<td>17.4</td>
<td>173.6</td>
</tr>
</tbody>
</table>

FHIA-03, FHIA-18 and SH-3436 from the Fundación Hondureña de Investigación Agrícola (FHIA) have been introduced and studied. These have shown different levels of resistance to BS, resulting in a longer period of symptom evolution, a reduced production of inoculum (Table 1), and a high yield potential without protection by fungicides (Hernández and Pérez 1996).

After A. Hernandez y L. Pérez 1996.

* In more of the 50% of samples the spots development stops at stage 4 and 5.

Implementation of a bioclimatic warning system for timing fungicide treatments

Cuban climatic conditions are characterized by two well-defined seasons. One season is dry and cold (from mid November to April), with records of minimum absolute temperatures in the range of 7 to 18 °C, maximum in the range of 17 to 27 °C and rains dependent on the entry of cold fronts. The other season is rainy and hot (from May to mid November), with minimum temperatures in the range of 17 to 24 °C and maximum between 30 and 34 °C, and during which 85% of the 1200mm mean annual rainfall falls. A network of climatic and phenologic recordings has been established in the most important banana plantations. Temperature as weekly speed sums (thermophysiologic method of Livingstone) (Pérez and Mauri 1992, Pérez et al. 1993a, Porras and Pérez 1996),
weekly evaporation Piche and duration and quantity of rainfall accumulated during two weeks (Figure 2 and 3), are used together with weekly assessments of the stage of evolution of the disease for timing fungicide aerial treatments (Pérez et al. 1993a, Pérez et al. 1995, Pérez et al. 1998 unpublished).

In the last five years, the system (Figure 4 and 5) has allowed the total number of fungicide treatments to be reduced to 13 - 15/year in bananas and to 9 - 11/year in plantains. Fungicides in oil mixtures must inhibit the micelial growth inside the leaves as well as the further development of the early stages of the BS lesions to mature necrotic spots and ascospore production (Pérez 1993a, Pérez et al. 1995, Pérez et al. 1998 unpublished).

Use of oil alone and oil in water emulsion mixtures with fungicides

The fungicides in use (Pérez 1993, Pérez et al. 1993c) include triazole (14 $\alpha$ - demetilase inhibitors - DMIs); propiconazole (Tilt 250 EC, 100g a.i./ha); tebuconazole (Folicur 250 EC, 100g a.i./ha); hexaconazole (Anvil 250 EC, 100g a.i./ha); bitertanol (Baycor 250 EC, 150g i.a./ha); cyproconazol (Bialor 250 EC, 80 g i.a./ha); epoxiconazol (Opus 125 CS, 80 g i.a./ha); triadimenol (Bayfian 250 EC, 100 g i.a./ha); morpholine (tridemorph, Calixin 750 EC 450g a.i./ha) usually in 12 l of oil alone; benomyl + mancozeb (0.15 + 2.0 kg a.i.).

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Monitoring M. fijiensis population sensitivity to fungicides

Monitoring is carried out at least once in a year (optimally twice, at beginning and end of the rainy season), to detect shifts in the sensitivity of M. fijiensis population to benzimidazole and DMI fungicides (Pérez y Batlle 1993). No evidence of shifts in sensitivity to DMIs leading to a lack of control in the field has been observed up to the present. A shift towards reduced sensitivity to propiconazole was determined in M. muscolo populations from areas were Sigatoka disease control basically relies on propiconazole and tridemorph treatments (benzimidazole...
fungicides being excluded due the high level of resistance to benomyl, Mulf

towards reduced sensitivity to propicona-
zone was characterized with the lost of the rapid ac-
tion of propiconazole treatments on the Sigatoka evolution, but without a corre-
sponding effect on the level of control of the disease in the field, or on the length of fungicide activity. In spite of this, the shift in sensitivity has been slower than that reported in commercial banana plantations in Central America. Due to the poligenic nature of the resistance to the DMI fungicides, shifts in sensitivity to this group have been established by periodic monitoring. The monitoring of population sensitivity is preferably carried out in the same reference fields and related to previously established sensi-
tivity base lines or to reference popula-
tions coming from untreated fields, lo-
cated in areas far enough from treated fields to consider that there are no popu-
lation exchange between them (Pérez et al., 1985, Pérez y Batlle 1993).

Resistance to benzimidazole fungicides in M. muscula populations is widespread in the major part of the ba-

nana plantations in the country (Pérez et al. 1985). These populations are very stable and after more than ten years of not using benomyl, in some areas we

are stable and after more than ten years of

et al. 1985). These populations are very

in most of these areas, reintroduction of no more of two benomyl treatments/year has been considered wherever My-
cosphaerella spp. ascospores populations show a tolerance below 10 mg/ml of active ingredient in ascospore germi-
nation tests.

Conclusions

An integrated management programme for black Sigatoka disease control in ba-
nana and plantain has been successfully put in place in most of the banana plan-
tations in the country. This programme uses a warning system based on biocli-
matic parameters and its implementa-
tion has led the following achievements:

• a reduction in the contamination of the environment due to frequent fungicide sprays;

• a reduction of the risks of selecting fungicide resistant pathogen popula-
tions, due to the reduced and al-
ternated use of fungicides thus reducing selection pressure, and to periodic monitoring of the sensitivity of popula-
tions to the products;

• good disease control, as demonstrated by 10-12 healthy leaves at flowering and more than eight healthy leaves at bunch harvest.

The system can be used wherever weather conditions during different peri-
dods of the year allow an increase in the intervals between fungicide treatments, and where a minimal logistic and techni-
cal infrastructure allows treatments to be carried out in a short time period and where general sanitation measures can be established. ■

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Black Sigatoka confirmed in Brazil

Zilton José Maciel Cordero, Aristoteles Pires de Matos and Sebastião de Oliveira e Silva

Black Sigatoka disease, caused by the fungus Mycosphaerella fijiensis Morelet (perfect stage), Paracercospora fijiensis (Morelet) Deighton (anamorphic), is the most serious banana disease world-wide. Yellow Sigatoka, similar to black Sigatoka, has been present in Brazil since the 1940’s. Despite its severity, yellow Sigatoka is usually replaced by black Sigatoka in banana-growing areas where both diseases are present. This is due to the high aggressiveness of black Sigatoka, in comparison with yellow Sigatoka, thus inducing significant yield losses that can be as high as 100% if no control measures are implemented. Another direct effect of black Sigatoka is the increase in production costs due to the intensive spraying of fungicides needed to control the disease; in Central America the number of sprays may be as high as 40 per year, that is four times as many as the number of fungicide sprays used to control yellow Sigatoka. In addition, M. fijiensis is capable of infecting a large number of banana and plantain varieties, which are widely cultivated by small growers in the North and Northeast Regions of Brazil. In February 1998, José Clério Resende Pereira, Luadir Gasparotto and Ana Fabiola da Silva Coelho, plant pathologists from EMBRAPA/CPAA, detected black Sigatoka in Brazil. They observed a severe outbreak of the disease in several banana cultivars in the State of Amazonas and consequently notified the Brazilian Ministry of Agriculture. Banana experts from EMBRAPA/CNPMT visited several infected plantations and confirmed the presence of the disease in the Amazon region. Black Sigatoka incidence was particularly high in the banana cultivars Prata Anã and Macã and several local plantain cultivars; no infection was observed in the cultivar Ouro. Black Sigatoka disease was first detected in Brazil in banana plantations located near the town of Tabatinga, in the State of Amazonas, on the border with Colombia. The disease has already spread over more than 400 km from the initial detection site so that it is now present in banana-planting areas in the districts of Benjamin Constant and Coari, as shown in Figure 1.

Due to the destructive potential of black Sigatoka, it has been suggested that the following actions be immediately implemented.

Genetic Control
The first measure proposed is to replace the varieties susceptible to black Sigatoka, presently grown in the affected area, by resistant genotypes. Besides being the most economic and effective control measure, growing resistant cultivars is also an environmentally safe and sustainable technology. In August, to carry out this task, EMBRAPA/CNPMT will be providing 50,000 vitroplants of propagation material of both ‘Caipira’ and PV 03-44, both resistant to black Sigatoka. Other genotypes, such as FHIA-01, FHIA-03, FHIA-20, FHIA-21, SH-3640 and Thap Mæo, introduced and/or selected at EMBRAPA/CNPMT and showing resistance to the disease, will be multiplied in vitro to be subsequently tested in the Amazon Region. It is expected that planting resistant genotypes will decrease the inoculum potential, thus reducing yield losses, and will slow down the spread of the disease within the Amazon Region, as well as towards other banana-growing areas in Brazil.

Monitoring the spread of the disease
The Brazilian Ministry of Agriculture, through its Department in the State of Amazonas, has already decided on actions aiming at preventing/regulating transportation and commercialization of plants and plant products belonging to the Musaceae family, in order to prevent the spread of the disease.

Researchers and agronomists from EMBRAPA and the Ministry of Agricul-
ture will carry out surveys in banana-growing areas of the Amazon region. Such an activity will enable sanitary barriers to be established in order to prevent the movement of black Sigatoka infected material.

Possible impact on the Brazilian banana industry
Since most of the banana producers in Brazil are small and medium growers, who use very low-production technology and could not afford the cost of chemically controlling black Sigatoka, and given the disease severity observed in the Amazon region, it is thought that this will have a very negative impact on yield and fruit quality in areas where no control measures have been taken. For those growers who are used to controlling yellow Sigatoka, they will see an increase in production costs, since more intensive fungicide spraying will be necessary. In North and Northeast Brazil, it is expected that this will have great impact on plantain production since plantain cultivars are resistant to yellow Sigatoka, while highly susceptible to black Sigatoka.

The authors work at EMBRAPA/CNPMT, Rua EM- BRAPA s/n, Caixa Postal 007, Cruz das Almas, Bahia 44380-000, Brazil.

Report on black Sigatoka status in Venezuela in 1997

Gustavo Martínez, Rafael Pargas, Eduardo Manzanilla and Daniel Mu

Black Sigatoka caused by the fungus Mycosphaerella fijiensis Morelet is considered to be one of the most serious diseases of the genus Musa. The losses it causes have important socio-economic consequences in numerous countries in Latin America where bananas and plantains are a staple food. The symptoms of the pathogen consist of plant leaf necrosis causing a reduction in photosynthetic activity and, in parallel, a decrease in the quantity and quality of production and even the death of the plant (Gauhl 1994, Pérez...
1996, Stover 1984). Control of the disease based on the use of chemicals is possible but extremely expensive and hence small and medium-scale growers are the most affected.

The disease was detected in Venezuela for the first time in 1991 south of Lake Maracaibo, where it caused a drastic reduction in the production of plantains and dessert bananas (Martínez 1996). From then onwards, the transfer of plant material from this zone to other parts of the country was reduced; information campaigns, discussions with growers and experiments were organized so that growers could be informed on how to manage the disease rather than how to eradicate it. In spite of this, the spread of the disease in Venezuela could not be prevented.

It was reported in 1992 and 1993 in the Monte Andino piedmont (in Trujillo, Tachira and Merida states) and in Barinas state. It was subsequently observed in Yaracuy state in 1994 and in Carabobo and Aragua in 1996 (Martínez 1996).

In the face of this situation, the climatological data for each zone were analysed separately to establish the pathway of the spread of the pathogen and to identify the zones with a high risk of contamination up to 1996. It was thus demonstrated that the aggressiveness of the pathogen is directly related to climatic conditions; the zones most severely affected by black Sigatoka are characterised by precipitation of more than 1,400 mm per year and over 80% relative humidity. It was also noticed that black Sigatoka has not so far caused significant damage in Aragua state, which accounts for 50% of Venezuelan banana production. Precipitation there totals 1,100 mm per year spread over six months of the year and relative humidity is 74%. It should also be considered that chemical control of yellow Sigatoka is commonly performed in this zone and a high level of technology is used (Martínez 1996).

With the spread of the disease, the developments forecast in the 1996 report published in INFOMUSA (Vol. 6, No. 1: 16-17) are now taking place. In 1997, the disease was reported in May in Miranda state, in September in Monagas state and in November in Bolivar state. The Amacuro Delta and Amazonas state are considered as high-risk zones as the conditions are very suitable for the spread of the disease (see map).

It can thus be seen that the rate of spread of the pathogen has increased considerably. After taking five years to move from the west to the centre of the country, it took only one year to spread from the centre to the west. Amazonas state at the frontier with Brazil is now threatened. There, bananas are grown by indigenous populations and are an essential part of their diet in a fragile ecosystem in which the use of chemicals is not recommended. Disease-resistant or tolerant clones should therefore be grown.

However, it must be admitted that the presence of black Sigatoka in the country has caused radical changes in the way in which bananas are grown and all the efforts made in information campaigns have helped to achieve positive changes. Practices such as the elimination of the lowest necrosed leaves are rare as banana is considered as a subsistence crop.

From the research point of view, trials are in progress to evaluate the behaviour of disease-tolerant cultivars and the effectiveness of specific spatial arrangements allowing minimum competition between plants and the optimal use of means of production with the aim of achieving the most satisfactory yield possible. Cultural practices combined with fungicide applications according to the climatic conditions and in line with integrated management of the disease are currently being evaluated to establish technological points of reference for the future.

References

The authors at based at the Centro Nacional de Investigaciones Agropecuarias (CENIAP), Instituto de Investigaciones Agrícolas, Musaceas, Zona Universitaria, UCV. Apartado 4653. Maracay, Venezuela.
This very attractive brochure presents information on all the aspects of the crop: origin, diversity, economic importance, role in food security, commercialization, pests and diseases, research, processing.

**Genetic variability in wild bananas including Calcutta 4**

This contribution refers to an article by Rodomiro Ortiz and Dirk Vuylsteke in INFOMUSA 3(1):21 of June 1994, on “Tri-partite segregation ratios and genome differentiation in AAB plantain”. If the reaction is long delayed, this is because of preoccupations at the time of my moving out from Brazil. N.W. Simmonds should also have entered the fray himself before now; either he has not seen the material or he has given up trying to set the record straight.

**Les bananes**

A 16-page booklet, prepared by CIRAD in collaboration with INIBAP for the 1998 Paris Agricultural Fair is available at INIBAP Headquarters in French only.

**Musaforum**

Letters from readers are published by INFOMUSA in their original language.

**Books, etc.**

Routine post-harvest screening of banana/plantain hybrids: criteria and methods.

INIBAP Technical Guidelines 2.

B. E. Dadzie and J.E. Orchard

ISBN: 2-910-810-22-4

This is the second issue of a new series of trilingual (French, English and Spanish) technical guidelines published by INIBAP. This 76 page-book describes the key post-harvest criteria and methods/procedure for routine screening of new Musa hybrids. It is the result of a collaborative research project involving FHIA, INIBAP and NRI, and funded by ODA (now DFID).

Most of the methods and procedures described are simple, easy to use and require limited and inexpensive technology. The manual is designed to provide useful information to assist breeders and researchers in post-harvest selection of new Musa hybrids and to facilitate technology transfer. A first edition was published in English under the title “Post-Harvest Criteria and Methods for Routine Screening of Banana/Plantain Hybrids”.

French, English and Spanish versions are available on request from INIBAP headquarters.

Technical guidelines No. 3 “Evaluation of Musa germplasm for resistance to Sigatoka diseases and Fusarium wilt” are in press.

Ken Shepherd, Rua do Maçarico 20, 1 Esq., Quinta da Bicuda, Torre, 2750 Cascais, Portugal.
Dear Sir,

In the last INFOMUSA, de Oliveira e. Silva et al. stated that Prata Anã does not belong to the Prata (=Pome) subgroup. Shepherd (1990) has also noted previously that it is “taxonomically distinct”. However, Shepherd (1993 pers. comm.) has “had some doubts as to the distinctness of Prata Anã from the Pome subgroup — it seems to be similar in so many characteristics”.

I have been evaluating the Prata Anã accessions Santa Catarina Prata (ex Hawaii), Icecream (ex Cook Islands, this is not Icecream of Hawaii which is Blue Java/Ney Mannan) and Prata Anã (ex INIBAP Transit Centre, Belgium) during the last 10 years at South Johnstone. We obtained some tall variants (genetic off types) from the in vitro multiplication of Santa Catarina Prata on a number of occasions which were very similar to what we know in Australia as the old type Lady Finger (Pome) and essentially the same as the clone Brazilian I have seen in Hawaii. It is highly unlikely that these tall variants were due to any sort of mixup in the tissue culture laboratory.

Our old type Lady Finger is presumably much the same as Prata of Brazil (Brazilian?) and our improved Lady Finger is likely equivalent to Pacovan (of Brazil).

Prata Anã literally means Silver Dwarf (Prata ± Silver, Anã ± Female Dwarf) so in the naming of the varieties at least, there is no suggestion that there is any difference between Prata and Prata Anã except in the plant stature.

Due to the occurrence of these tall variants and the numerous similarities with Lady Finger (Pome) varieties, especially taste, I am surprised that Prata Anã should not belong to the Pome subgroup.

Because other folk would be interested, this matter needs airing in INFOMUSA. Why do de Oliveira e. Silva et al. consider Prata Anã not to belong to the Pome subgroup?

Reference

Jeff Daniells, QDPI, Box 20, South Johnstone, 4859 QLD, Australie.

INIBAP News

Recruitments
Dr Agustin B. Molina Jr has recently been recruited by INIBAP to replace Dr Ramon Valmayor as Regional Coordinator for Asia and the Pacific. Gus, as he is generally known, is a citizen of Philippines and obtained his Ph.D. in plant pathology from Pennsylvania State University in 1983. He has 10 years experience of banana production in Latin America, where he worked as a scientist, research manager and technical adviser. He also has extensive experience in teaching and training in plant pathology. Before joining INIBAP, he was associate professor and chairman of the Department of Plant Pathology at the University of the Philippines at Los Baños, Gus is fluent in both Spanish and English.

Gus has now taken over from Ramon Valmayor at the INIBAP Regional Office located in the Philippines, but will continue to remain in close contact with Ramon, who is now an INIBAP Honorary Research Fellow. INIBAP would like to take this opportunity to welcome Gus and to wish him every success in his new position.

Thomas Moens, a Belgian citizen, has recently been recruited for the position of Associate Expert in nematology to replace the position formerly held by Nicole Viaene at FHIA, Honduras. Thomas, who is based at the Corporacion Bananera Nacional (CORBANA), Costa Rica, took up his duties in June. Thomas will dedicate part of his time to...
provide a nematological evaluation component to the banana research programme of CORBANA. He will also work on the nematological evaluation of one of the segregating populations to be established at CORBANA by INIBAP, as well as evaluating parental lines of breeding programmes and wild species as potential sources of resistance to Radopholus similis.

Stjin Messiaen, a Belgian citizen, has been selected for the position of Associate Expert in entomology. He will be based at CRBP in Cameroon, where he will carry out research towards the development of integrated control strategies for the banana weevil borer Cosmopolites sordidus. Stjin will take up his duties in July.

Thomas and Stjin are funded by the Vlaamse Vereniging voor Ontwikkelingssamenwerking en Technische Bijstand (VVOB), Belgium. INIBAP would like to take this opportunity to welcome them and to wish them every success in their new positions.

Emmanuel Gonnord, a French citizen, was recently selected for the position of Accounting Assistant at INIBAP Headquarters. Emmanuel has seven years of experience in accounting. He is fluent in English. His main task will be to assist Tom Thornton, the INIBAP Financial Manager. Emmanuel will take up his duties on 17 August.

Régine Roux, who has worked for INIBAP for 10 years, and was one of INIBAP's first employees, is to leave at the end of June. During her time with INIBAP, Régine provided valuable secretarial support to various staff members. In particular, she worked for three years as Programme Assistant to Dr David Jones and since 1992 has been working with Tom Thornton. INIBAP would like to take this opportunity to wish her every success for the future.

Information activities

LACNET Regional Information Network
INIBAP signed a Memorandum of Understanding with IICA-UPEB on 14 January 1998 aimed at encouraging the exchange of information between the LACNET regional information and documentation network members and IICA-UPEB. In the framework of this MoU, the IICA-UPEB banana and plantain information and documentation service (SIDBAP) agrees to give free access to the bibliographic abstracts contained in its database SIBBANA to the national centers which are members of the regional network. From their side, the national centers will update SIBBANA by regularly providing new information as it becomes available in their countries. Bilateral agreements regarding exchange of documentation will be established between SIDBAP and participating centers.

INIBAP participates in the project Agri2000 managed by IICA, Costa Rica, which aims to assemble and make available online various bibliographic agricultural databases. INIBAP provided to IICA a bilingual version (English, Spanish) of MUSA-LIT, which is searchable at the following address: http://www.iica.ac.cr/espanol/. The SIBBANA database managed by IICA-UPEB is also available through this address.

Banana thesaurus

Nitzia Barrantes, former Head of the UPEB Information and Documentation Center (UPEB-CID), recently completed the revision and updating of the Spanish version of the “Tesauro del Banano” (banana thesaurus), published by UPEB in 1983. A thesaurus is a specialized, controlled and structured vocabulary, which aims to facilitate the analysis and retrieval of information on a specific topic. This new version of the thesaurus includes 21 thematic areas covering all aspects of Musa. Thanks to the collaboration of a computer scientist, Gilda Ascensio, who worked closely with Nitzia, the new edition of the thesaurus is computerized and can be consulted on screen in Spanish. The software is very user-friendly and allows the thesaurus to be linked to any relevant database, thus facilitating the indexation of documents (in information science, indexation is the process of selection of keywords which permit a document to be identified) and the retrieval of information. INIBAP recently recruited a student who is presently translating the thesaurus into French and English. A trilingual version
will be soon available for the users of the INIBAP databases MUSALIT and BRIS.

Can you help us to illustrate INIBAP publications

INIBAP is presently reorganizing and computerizing its photo library. With the aim of making its publications more attractive and accessible, INIBAP would like to enlarge its slide collection. For this reason, INIBAP encourages all persons who possess original and good quality slides on Musa (diseases, pests, markets, field production, etc.) and who would like to share them with INIBAP, to send duplicates (or originals that INIBAP could duplicate) to INIBAP Headquarters, attn: Claudine Picq. For each slide sent, details should be provided of the author of the photograph, the place where it was taken and a comprehensive caption should be added. In return, INIBAP will give credit to the author for every slide published. We thank you in advance for your cooperation.

Reprint of Banana descriptors

Due to the high demand for the “Descriptors for bananas, Musa spp.”, this publication has been reprinted in English and copies are available at INIBAP Headquarters.

Public awareness activities

INIBAP participated in the 1998 Paris Agricultural Fair as a partner with CIRAD. The stand developed by CIRAD and INIBAP featured bananas, and it focused on informing visitors about the global importance and wide diversity of bananas and plantains. As the fair attracts over half a million visitors, this was an important public awareness event for INIBAP. In collaboration with CIRAD, INIBAP participated in the production of a range of public awareness materials, and was particularly involved in the development of an interactive multimedia display presenting, in an informal and attractive manner, all aspects of the crop. An eye-catching brochure about bananas was also produced and proved very popular with visitors to the stand. INIBAP’s activities were described on several posters and more than 2000 banana diversity posters, produced in collaboration with the I PGRI public awareness specialist were distributed.

Training courses and meetings

Training course in Cameroon

In December 1997, Ekow Akyeampong, INIBAP Regional Coordinator for Western and Central Africa, assisted in the organization of a training course on “Genetic improvement of Plantains” organized jointly with IITA and CRBP at Nyombé, Cameroon. Thirteen Musa scientists and technicians from Cameroon, Côte d’Ivoire, Equatorial Guinea and the Democratic Republic of Congo attended the course. Emile Frison, Director of INIBAP, gave a half-day lecture on detection of Musa viruses.

CORPOICA Seminar

Several INIBAP staff members attended the "International Seminar on Plantain Production" organized in Armenia, Colombia by CORPOICA, the Comité de Cafeteros del Quindio, INIBAP, SENA and the University of Quindio from 4-8 May 1998.

The objective of the seminar was to present a synthesis of 13 years of research on Plantain at ICA and CORPOICA, with the purpose of establishing a national research network on Plantain which would link Universities and other research entities.

The seminar was attended by more than 200 participants. Beside a massive participation of Colombian researchers and farmers, scientists from several Latin American countries made presentations. Noteworthy was the participation of four representatives of CRBP, Cameroon, providing a view on plantain research, development and production in West and Central Africa. Ben Lockhart (USA) and Marie Line Caruana (France) provided useful clarifications on the Musa virus situation, more particularly in the area of banana streak virus. Jean-Pierre Horry, INIBAP Genetic Resources Coordinator, made a presentation on the “Management of global Musa genetic resources through networking”. Franklin Rosales, INIBAP-LACNET Regional Coordinator, acted as Chairman of the session on Genetics and Breeding. Ramiro Jaramillo, former LACNET Regional Coordinator, was also present as a special guest. Sébastien Tripon, Associate Expert to LACNET, also attended this meeting.

During the meeting, Sylvio Belalcázar, Head of CORPOICA Plantain Programme, was honored by CORPOICA for more than a decade of dedication to banana research.

Emile Frison interacting with the participants of the workshop organized in West Africa.
plantain research; his official retirement from CORPOICA was announced effective June 30, 1998. Gerardo Cayon will replace him in this position.

The proceedings of this important meeting are already available. To obtain a copy, please contact CORPOICA, Apartado aéreo 1807, Armenia, Quindio, Colombia.

Following the Seminar, Jean-Pierre Horry visited the field genebank of El Agradable in order to verify the classification of genotypes in the field. All material delivered from ITC appears true to type, except cv. Pisang Lilin that showed an unusual shape for the male bud, already known from several other locations. Jean-Pierre also followed up on the implementation of the Musa Germplasm Information System software.

Towards a sustainable banana economy

Emile Frison, Director of INIBAP attended the International Banana Conference which was held in Belgium from 6-8 May. An International Banana Charter was released by the organizers of the conference, setting out proposed sustainable guidelines for the banana industry worldwide. At a press conference to launch the Charter, the Coordinator of EUROBAN (the European Banana Action Network), John Daly, stated, “There will be no future for the banana industry unless everyone, companies, unions and governments work together. The Charter is not an ultimatum, but a way to move beyond the traditional banana wars which have been raging internationally for years.” The International Banana Charter calls for prices that reflect real costs at every stage of production. Consumers must pay a price that covers the social and environmental costs of banana production. The Charter also calls for clauses to be included in the World Trade Organization (WTO) agreements, which guarantee basic social and environmental standards.

The three day conference in Brussels brought together 300 people from 45 countries representing the key players in the banana trade including banana workers, small farmers, producers, companies, international institutions, policy makers and scientists. Those attending the meeting heard evidence of problems related to the heavy use of pesticides in commercial banana production, while Caribbean representatives presented a plea for action to be taken to ensure their future within the banana sector. A presentation by Dr Emile Frison highlighted the important role that research on the genetic improvement of bananas can play in the development of sustainable systems of production and the need for further resources to be directed towards such research.

This meeting was the first time that such a large number and wide range of stakeholders in the export banana sector had been brought together to discuss the major issues facing the industry.

Second training workshop on MGIS

The second regional training workshop on the Musa Germplasm Information System (MGIS) will take place in Brisbane, Australia from 6-11 July 1998. The main objective of the course is to provide training in MGIS methodology to the curators of the collections maintained by institutions already involved in MGIS activities in the Asia/Pacific region. The principal trainers will be Elizabeth Arnaud and Jean-Pierre Horry.

Bananas and food security

People who wish to attend the international symposium on Bananas and food security, to be held in Cameroon from 10-14 November 1998, should register before 30 September. Registration fees are US$100 before 15 August and US$150 after this date. Registration payment should be sent by cheque in US dollars to Symposium Secretariat, CRBP, BP 832, Douala, Cameroon or by bank transfer to: “CRBP-Symposium 1998”, Account Number 30 904 188 3716 Q - SCB-Crédit Lyonnais, Agence Centrale Entreprise, BP 300, Douala, Cameroon.

More information on this important event was published in a leaflet attached to INFOMUSA Vol. 6 No. 2. The leaflet is available on request in French and English at INIBAP Headquarters.

IMTP Impact assessment study

As part of IPGRI’s contribution to the CGIAR Impact Assessment and Evaluation Group’s activities, IPGRI is conducting an in-depth study of INIBAP’s IMTP. The study has involved a survey of IMTP evaluation site managers and managers of national evaluation programmes using questionnaires developed in collaboration with IPGRI’s impact assessment specialist. The results of the survey are presently being analysed by IPGRI and a full report will be published shortly. Initial results indicate that IMTP has been successful in achieving its original objectives and has evolved appropriately according to the needs of participants. One output of the study has been further information on the performance of the FHIA hybrids recommended for further evaluation as a result of IMTP Phase I. Although in many countries these hybrids are still only being grown in experimental sites, many countries reported favourable initial reactions to the hybrids by farmers. The study also revealed that the lack of mass propagation facilities is a major constraint to the wide-scale distribution of improved varieties to farmers in many countries.

Visit by head of CGIAR

Dr Ismail Serageldin, the Chairman of the Consultative Group on International Agricultural Research (CGIAR) and Vice President of the World Bank, visited INIBAP during a recent visit to Montpellier AGROPOLIS. After a presentation of the INIBAP programme, discussions were held on the progress of PROMUSA and role of the World Bank in the Banana Biotechnology Consortium.
The International Plant Genetic Resources Institute (IPGRI) is one of the sixteen international agricultural research centres supported by the Consultative Group on International Agricultural Research. In 1994, the International Network for the Improvement of Banana and Plantain (INIBAP) became a programme of IPGRI. The objective of the INIBAP programme is the improvement of smallholder banana and plantain production. IPGRI has its headquarters in Rome and has a total staff of more than 150, including 40 internationally recruited professionals, located in 15 countries. Funds for IPGRI’s research agenda are provided by 48 governments and a number of development assistance agencies. The INIBAP programme has its headquarters in Montpellier, France and regional offices in various banana-producing countries. Its global activities are implemented at the regional level through regional networks.

IPGRI is seeking well-qualified candidates, with proven ability to work effectively with people from widely different national and cultural backgrounds, for a scientific position to be located at the INIBAP headquarters in Montpellier, France:

**Musa Genetic Resources Scientist**

The appointee will be the focal point for INIBAP activities related to Musa genetic resources. He/she will be responsible to the Director of INIBAP and will work in close consultation with the INIBAP staff at headquarters and other staff at IPGRI headquarters and in the regions. The main responsibilities will be to manage a global project on the conservation and use of Musa genetic resources and to promote and coordinate research activities in related areas. The work will involve close collaboration with research programmes on Musa worldwide. The appointee will be required to provide technical support and advice to national programmes of producing countries in the areas of his/her expertise and to contribute to the production of various publications resulting from or related to the project.

Potential appointees will have a higher degree (preferably at doctoral level) in a relevant field of biology or agriculture, extensive experience in research on Musa, preferably in the area of genetics, and a good knowledge of Musa diversity. Experience in banana agronomy is an advantage. They will also have experience in research coordination as well as outstanding communication (oral and written) and interpersonal skills. In addition, they should have demonstrated experience in project formulation and management and in working in developing countries. Candidates should be fluent in English (both oral and written) and possess good computer skills. Knowledge of French or Spanish is an advantage.

**Further information:** Dr Emile Frison, Tel: +33-4-67 61 13 02, fax +33-4-67 61 03 34, e-mail: e.frison@cgnet.com

**Terms and conditions:** Initial appointments will be for a period of three years. IPGRI offers an internationally competitive salary and benefits package and assistance with relocation expenses.

**Applications:** Should include a letter of application, a full curriculum vitae and the names, addresses, e-mail and/or fax numbers of at least three referees. Applications should reach INIBAP no later than 15 September 1998 and be addressed to: Dr Emile Frison, Director, INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France.

IPGRI is an equal opportunity employer and strives for staff diversity in gender and nationality.
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Instructions to authors

Typescripts should be prepared in English, French or Spanish and submitted in duplicate to the Managing Editor. They should be double-spaced throughout. All pages (including tables, figures, legends and references) should be numbered consecutively. Include the full name of all the authors of the paper, together with the addresses of the authors at the time of the work reported in the paper. Indicate also the author nominated to receive correspondence regarding the paper.

If the typescript was prepared on a computer, please send a copy on diskette (or by e-mail) along with the printed ones, indicating the name and version of the wordprocessor used.

Abstracts
An abstract not exceeding 200-250 words should be sent in the same language as the typescript, as well as translations (including the title) into the two other languages, if this is possible.

Acronyms
These should be written in full the first time they appear in the text, followed by the acronym in parenthesis.

References
All literature references made in the text should be referred to by author(s) and year of publication (e.g.: Sarah et al. 1992, Rowe 1995). A list of references, in alphabetical order, should be provided at the end of the text. Please follow the style shown below:

Periodicals:
Books:

Illustrations
These should be numbered consecutively and referred to by these number in the text. Each illustration should include a clear and simple caption. Graphs: provide the corresponding raw data with the graphs. Drawings: provide originals if this is possible. Black and white photographs: provide them on bright paper and with good contrast. Colour photographs: provide good quality proofs and films or original slides.

Note: When plant material used for the experiments reported originates or is registered in the INIBAP genebank, its accession number (ITC code) should be indicated within the text or in a tabular form.

Thank you in advance for following these instructions. This will facilitate and accelerate the editing work.
The following publications are available from headquarters:

INIBAP/IPGRI/CIRAD 1996. Descriptors for Banana (Musa spp.).

The following publications are available from ASPNET: