The case for growing beer bananas
A new method for producing cell suspensions
Mycosphaerella culture
Results for reader survey
INIBAP is 20 years old: how it all began

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The mission of the International Network for the Improvement of Banana and Plantain is to sustainably increase the productivity of banana and plantain grown on smallholdings for domestic consumption and for local and export markets. INIBAP is a network of the International Plant Genetic Resources Institute (IPGRI), a Future Harvest centre.

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A new IPGRI home for INIBAP

As part of a major re-appraisal and re-structuring of its strategy, IPGRI launched the ‘Commodities for Livelihoods’ Programme, which will draw together the existing work on cacao, coconut, and banana and plantain. INIBAP retains its name and identity, which is only fitting for an organization turning 20 this year, and will continue to work through existing partnerships and projects, while carry on developing new ones. The new programme presents an opportunity to share experiences across commodities and develop a more effective approach to use the diversity of these crops to improve the livelihoods of people in developing countries.

Before the new structure became operational in January 2005, IPGRI commissioned an external review of its commodity work. The Centre-Commissioned External Review (CCER) looked back at the activities that had been carried out on the three commodities independently from March 2000 to the end of 2004. The review also looked forward to the integration of the three commodities within the programme. A stakeholder’s survey also played an important part in informing the review panel about our partners’ perspective on our work. We would like to take this opportunity to thank all those who took the time to answer the survey. The main results are presented in the Focus on section.

The review panel commended IPGRI’s work on the three commodities and made a number of recommendations on the scope and role of the new programme and on how to maximize the benefits of networking, which is central to achieving our objectives. The panel also commended INIBAP’s information products. Like the majority of readers who answered our survey (see Focus on section), the panel praised INFOMUSA, but differed from them in recommending that INFOMUSA should not be transformed into a peer-reviewed journal. It recommended, however, that INIBAP establish a system to verify the accuracy of the information published in INFOMUSA.

This recommendation sits well with our desire to achieve basic standards of credible scientific reporting while keeping INFOMUSA accessible to authors who, for lack of financial or institutional support, find it hard to publish their work in peer-reviewed journals. Rather than refuse papers outright, we prefer to work with the reviewers and authors to improve the quality of the submitted papers. Even when we decide to reject a paper, we try to offer advice on what needs to be improved, should the authors want to redo the experiment or submit a revised version elsewhere.

Critical review by fellow researchers is one of the most important means by which erroneous hypotheses are challenged and research methods improved. If INFOMUSA were to become a peer-reviewed journal, a considerable body of research carried out on Musa would no longer be exposed to this form of scrutiny. Progress might well be slower as fewer people would be able to go public with their results and fewer ideas would be shared and tested against the opinions of others. We urge you to make full use of the Forum section, which we introduced to allow readers to offer constructive criticism and to debate the issues arising, as occurred recently over the question of planting depth.

Many of the readers who answered the INFOMUSA survey would like to see more articles on applied research. The Focus on section is currently the only part of INFOMUSA for which we solicit articles and we do so because we receive so few submissions for this new section. If you really do want to see us guide the content of other sections by soliciting articles on more applied topics, for instance, then we can test the feasibility of this idea. In the end, INFOMUSA is your journal, so whether it is a commentary, a scientific or technical article, or a letter to the editor, keep sending your contributions and thank you to all of you who filled in the reader survey.

The editors
Why beer bananas? The case for Rwanda
S. V. Gaidashova, S. H. O. Okech, C. S. Gold and I. Nyagahungu

Rwanda is one of the major producers of bananas in the East African Great Lakes region and has one of the highest consumption rates. Bananas occupy 23% of the country’s arable land and contribute more than 50% of annual crop production in terms of fresh weight (Mpyisi et al. 2000). Banana is both a food and a cash crop for most producers and, as such, is a key component of Rwanda’s food security. It is also the primary source of income for farmers in some of the most productive agricultural zones in Rwanda. Most production is on small plots. A large number of clones are grown, including the local East African highland cooking and beer cultivars (AAA-EAHB) and introduced beer (AB, ABB) and dessert (AAA, AB) types. For the last 15 years, beer cultivars have dominated banana production (increasing from 67% to 71% at the expense of dessert types) (Food Security Research Project 2000).

The predominance of beer bananas is controversial. High population pressure makes land use and food production a key concern. Some people at the Ministry of Agriculture have advocated replacing beer bananas with cooking bananas and/or more nutritious annual food crops. Clearly, the factors that have influenced the spread and increased importance of beer bananas to the rural farming community need to be given serious consideration in policy decisions. In other words, do beer bananas hold a comparative advantage over other cash crops as a source of income to the rural poor? The economic and environmental consequences of replacing beer bananas with other cash crops have not been properly analysed at a time when the vision for a regional agricultural policy is increasingly emphasising marketable crops and income generating opportunities for small farmers. There is a need to balance the government’s agricultural policies with food security needs, rural household income, cultural values and environmental concerns.

To address some of these issues, the Banana Programme of the Institut des Sciences Agronomiques du Rwanda (ISAR) and the International Institute of Tropical Agriculture (IITA) conducted a participatory rural appraisal in the country’s key banana production zones. Its primary objective was to gain insight into the farmers’ perceptions of the relative profitability of the various crops available to them. The information generated from this study is being used in shaping the national banana research strategy.

Materials and methods
The participatory rural appraisal was conducted in November and December 2000 in three villages in each of the four major banana-growing regions of Rwanda — Cyangugu (in the southwest), Kibungo (in the southeast), Kigali Rural (in central Rwanda) and Kivu Lake Border (in the northwest) — for a total of 12 sites (Figure 1). The participatory rural appraisal was conducted by a multidisciplinary team, including specialists in banana production, socioeconomics and post-harvest. Activities included group and key informant interviews and on-farm visits.

At each site, the group interview was the primary means of collecting information. Regional agricultural extension officers liaised with local community leaders to assemble groups to meet with the survey team from ISAR to discuss agricultural issues. As such, the participants represented a cross-section of villagers and were not biased towards banana producers. Groups consisted of 30 to 154 farmers.

Group interviews were semi-structured and conducted in local language. The interview teams solicited opinions from as many farmers as possible to avoid certain individuals from dominating the discussion. Where necessary, a special effort was made to draw out the opinions of women. Wherever possible a consensus was reached. Where a consensus could not be reached, a show of hands was taken to reflect divergent opinions.

Participants were asked to list all the banana cultivars by use and type. They discussed the importance, advantages and disadvantages of each cultivar and the criteria they use to decide which cultivar to grow. To obtain the opinions of farmers
on the government’s policy of encouraging farmers to shift from beer bananas to cooking bananas, annual food crops or coffee, and how such a change might affect them, we first asked which crops had the best farm-gate price and which were most profitable. We then asked why they preferred beer bananas to cooking bananas, given that cooking banana bunches sold for more money. We also asked farmers how replacing beer bananas with other crops might affect them. Detailed site descriptions and methods are presented in Okech et al. (2004).

Results
Beer bananas are the most common cultivars in three growing areas: Kivu Lake (85 to 90% of cultivars), Cyangugu (60 to 80%) and, Kigali Rural (60 to 70%). In Kibungo, cooking bananas predominate whereas beer types represent between 20 to 30% of the cultivars (Figure 2). Dessert types never represent more than 10% of the cultivars.

The proportions of the different banana types within each region and at each site are influenced by market opportunities, dietary preference, food security and the relative performance of the cultivars across a range of criteria. Cooking bananas are widely viewed as poorly adapted to withstand stresses such as untimely rainfall, drought, declining soil fertility, intensive cropping systems (e.g. intercropping) brought on by land pressure and reduced management levels. Cooking bananas also require a well-developed market infrastructure because of their short shelf life and the lack of demand for processed products. As a result, cooking bananas are predominant only where they are the main staple food or in sites with good access to the major market of Kigali. In Kibungo, where soils tend to be more fertile (Lassoudière et al. 1989) and farmers have a good outlet for their cooking bananas in Kigali, cooking bananas predominate.

In contrast, beer bananas are considered more tolerant of adverse growing conditions and low levels of management than cooking bananas. Moreover, they are considered better suited to an underdeveloped market infrastructure because the beer they produce has a longer shelf life and the demand for that product is higher than the one for cooking bananas. Farmers also
say that introduced beer bananas (ABB) perform better on poor soils than AAA-EAHB bananas (both cooking and beer type). ABB and AB beer bananas do seem more efficient in producing on nutrient poor soils. Leaf nutrient concentrations published by Lahav (1995) show that ABB bananas have much lower plant nutrient concentrations (15% less N and 50% less K) than AAA cultivars. Similarly, Bosch et al. (1996) found much lower leaf nutrient concentrations (up to 23% less N and 50% less K) in the AB beer banana 'Kisubi'.

Farmers listed 14 criteria used to decide which cultivar to grow. They include bunch size (high yield) (12 sites), taste, as far as cooking and dessert bananas are concerned (12 sites), the quality of the juice produced by beer bananas (10 sites), marketability (7 sites), resistance to pests and diseases (7 sites), multiple uses (6 sites), plantation longevity (5 sites), finger size, as far as cooking bananas are concerned (3 sites), tolerance to poor soils (3 sites), maturation rate (3 sites), availability of planting material (2 sites), resistance to toppling (2 sites), the quality of the wine produced by beer bananas (1 site) and tolerance to intercropping, as far as cooking bananas are concerned (1 site).

**Marketing**

Across the study regions, 80% of the beer banana bunches are processed into beer at the farm level and sold to local consumers or intermediaries. The remaining 20% are sold to local brewers. Banana beer is common in Kigali, Cyangugu, Kibuye and Gisenyi. Gikongoro is the major market for the beer bananas and beer produced in neighboring Cyangugu. Some of the beer from the Kivu Lake border region is also sold in Kigali.

Kigali is the country’s major market for cooking bananas. Much of the bananas sold in Kigali are produced in the Kibungo and Kigali Rural regions. Cooking bananas produced in the Cyangugu region serve the smaller, but important, market in Cyangugu town, while farmers in Kivu Lake border region sell in markets in Kibuye and Gisenyi. There is little movement of cooking bananas from the Cyangugu and Kivu Lake border regions to Kigali, which is also the primary market for dessert bananas.

The farm-gate prices of a 20 kg bunch of cooking banana are between 800 and 3000 Rwandese francs (1US$ = 450 francs), while beer types sell for 100 to 800 francs. Prices tend to be higher in sites with developed market and road infrastructures (e.g. around Kigali). Despite the higher
prices paid for cooking bananas, farmers at nine sites reported that beer bananas are more profitable and, of all the cash crops, contribute the most to household income. Farmers attributed the higher profitability to the facts that: (1) bunch size requirements are less stringent for beer than for cooking and dessert bananas; (2) lower standards mean that beer bananas can do with less management and inputs and are thus cheaper to produce; (3) beer bananas can withstand marginal soils, drought and other adverse growing conditions and give appreciable yield throughout the year, thereby providing continuous income; (4) beer bananas are readily available and have wider market than other crops; (5) the processed products (juice, wine, and beer) add value to the crop and hence higher profit margins (the 11 bottles of beer that can be obtained from a 20 kg bunch would sell for about 1100 francs) and (6) the products can be stored for long periods, are more easily transported over longer distances and have lower transportation costs.

At the sites in Cyangugu, Kigali Rural and Kivu Lake border regions where beer bananas are important, farmers expressed an unwillingness to switch from beer bananas to cooking bananas or other crops. Firstly, cooking bananas require more fertile soils, higher levels of management and inputs, and a well-organised market and transport system. Producing cooking bananas entail greater risks and lower returns to farmers, especially those further away from Kigali. These disadvantages do not compensate for the higher prices paid for cooking banana bunches. Secondly, beer bananas are available throughout the year and allow for a better distribution of income than seasonal crops. For example, although maize might bring higher returns, its seasonality, sensitivity to weather fluctuations and need for greater levels of inputs make it less desirable. Cassava and sweet potato can withstand adverse production conditions, but the returns tend to be much lower than the ones for beer bananas, whereas the market for Irish potato, sorghum, and vegetables is very small. Thirdly, beer bananas are seen as preferable to coffee because the latter requires higher levels of inputs and has a disorganized local market structure, which farmers see as having little potential to absorb increased production and generate profitable margins. This situation contrasts with the one in Kibungo, where soils are more fertile and farmers are more open to change.

**Discussion**

The participatory rural appraisal confirmed that beer bananas predominate in Cyangugu, Kigali Rural and Kivu Lake Border regions, while cooking bananas are the most important crop in Kibungo, in agreement with Bart (1993) and Kangasniemi (1998). In recent years, the Ministry of Agriculture of Rwanda has expressed dissatisfaction with the importance of beer bananas because these are seen as taking up land that could otherwise be turned over to food production. Therefore, some members of the ministry advocated a drastic reduction in the area cultivated with beer bananas, in favour of cooking bananas and annual crops.

Our survey suggests that this will be difficult to achieve. From the farmers’ point of view, beer bananas remain the most appealing option. Nevertheless, we recommend studying the profitability of the different banana types to verify this opinion. It is possible farmers would find other types of banana more profitable if there were a more developed market for them and if the requisite infrastructure and market access were developed. However, farmers are not convinced about the government’s concerns over malnutrition, citing the availability of beans (commonly intercropped with banana) and other starchy staples (cassava and sweet potato).

Moreover, there is no evidence that farmers are particularly poor or have had problems with food security in regions where beer bananas predominate. Many farmers use the income from the sale of beer bananas to purchase beans, cassava, sweet potatoes and other sources of carbohydrate. By contrast, problems with poverty and hunger are most pronounced in areas where rainfall is low (e.g. Umurara and Bugesera in eastern Rwanda). These areas are marginal for many crops, including bananas.

There are also environmental aspects that need to be considered before recommending the replacement of beer bananas by annual crops. Erosion is a major problem in Rwandan and erosion from permanent banana plots is less than three times that of annual cropped plots (Lufafa et al. 2003).
Possible approaches to achieving the government’s wish to reduce the area on which beer bananas are grown would be: (1) to introduce high yielding disease resistant beer/juice cultivars and (2) to promote market research and planning to facilitate the transport from remote areas, like the Kivu Lake border region, to the lucrative market of Kigali and hence encourage the adoption of cooking bananas. In addition, farmers might take on other crops if they could be assured reliable markets. Better infrastructures would provide farmers with greater options to improve their livelihoods. Until these issues are addressed, however, we can expect many Rwandan farmers to continue relying on beer bananas.

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References


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Agronomic evaluation of production and quality of ‘Yangambi km 5’ (AAA) and ’Dátil’ (AA)

A. Vargas and J. A. Sandoval

The dessert banana ‘Yangambi km 5’ (AAA) is known in the Democratic Republic of Congo, its country of origin (Daniells et al. 2001a), as ‘Ibota’ meaning “many small fruits” (Daniells and Bryde 1995). The fruits have a slightly acid, pleasant flavour (Daniells and Bryde 1995, Menon 2000). Its resistance to black leaf streak (caused by Mycosphaerella fijiensis), Sigatoka disease (caused by Mycosphaerella musicola), the borer nematode (Radopholus similis), and possibly to banana borer weevil (Cosmopolites sordidus), has meant that this cultivar has been distributed to many countries for study and evaluation (Daniells and Bryde 1995).

The dessert banana ‘Dátil’ (AA) originates from Malaysia (Daniells et al. 2001b) and is commercially important in southeast Asia. The cultivar is called ‘Baby banana’ or ‘Lady finger’ in Costa Rica, ‘Pisang mas’ in Indonesia and Malaysia (Valmayor et al. 1990), ‘Sucrerie’ in Australia (Daniells 1986), ‘Bocadillo’ in Colombia (Buitrago et al. 1994) and ‘Titiaro’ in Venezuela (Haddad and Borges 1974). The flesh of the fruit is whitish-yellow, smooth, soft and sweet, and has a distinct aroma. The skin is very thin and the cuticle prone to bruising, making
transport and preservation difficult. Fruits achieve twice the price of better known types of banana. Despite the growing interest in Costa Rica of cultivating them for export, there is a lack of data on the agronomy and production of ‘Yangambi km 5’ and ‘Dátil’. The objective of this work was to evaluate the growth, production and fruit quality of this type of musaceae.

Materials and methods
The study took place at the Centro de Investigación Agrícola 28 Millas, Corporación Bananera Nacional (CORBANA S.A.). The experimental field was located in Limón province, Cantón de Matina, at an altitude of 25 metres. The investigation was carried out between September 2000 and October 2002, a period covering two production cycles. Up to 1990, the experimental site had been planted with pejibaye (*Bactris gasipaes* K.), also known as peach palm, to produce palm hearts and left unused thereafter. The soil texture is a clay loam (sand 35.8%, clay 34.9%, silt 29.2 %), with pH 6.2, extractable acid 0.23, organic matter content 2.2%, Ca 27.7 cmol/L, Mg 11.7 cmol/L, K 0.96 cmol/L and a cation exchange capacity of 40.4 cmol/L.

The planting materials were corms of the cultivars ‘Dátil’ and ‘Yangambi km 5’ weighing between 1 kg to 3 kg. The materials were arranged in a randomized complete block design with six replicates or plots. The usable part of the experimental plot contained 12 plants arranged in double furrows spaced 2.75 m apart, with 1 m between the rows of the double furrow and 2.15 m between the plants of the same furrow, for a density of 2480 plants/ha. Fertilizer, 0-46-0 (N-P$_2$O$_5$-K$_2$O), was applied at 22 g per plant one month after planting and then monthly at 47 g per plant of 15-3-31 (N-P$_2$O$_5$-K$_2$O) until the end of the study.

Chemical treatments to control black leaf streak were not applied. Sampling for nematodes was performed on the suckers of recently flowered plants in the second production cycle. Sampling was repeated on two occasions on different groups of plants. The first sampling was on 9 plants for each cultivar and the second on six plants for each cultivar. The damage by larvae of the banana borer weevil was estimated on recently harvested plants of the first (68 and 41 plants) and second (68 and 31 plants) production cycles of ‘Yangambi km 5’ and ‘Dátil’ respectively according to the method described by Villardebo (1973).

The bunch was bagged 15 days after emergence of the inflorescence. Only the false hands were removed. The bunch was harvested 10 and 8 weeks after flowering for ‘Yangambi km 5’ and ‘Dátil’ respectively. The plants were not propped up.

The following agronomic parameters were measured: days to flowering, height (measured from the base to the point where the last two emerged leaves overlapped), girth (measured on the first third of the pseudostem, using a vernier caliper), number of leaves of the motherplant at flowering and at harvest, number of days from planting to first flowering, number of days from the first to second flowering, height of the sucker, number of foliage leaves on the sucker, weight of the bunch and rachis, number of hands and fruits per bunch; diameter of the middle fruit of the second, fourth and sixth hands, and length of the fruit of the middle fruit of the second, fourth and sixth hands.

The parameters pertaining to the postharvest quality of the middle fruit of the cluster’s outer row were Brix, flesh firmness and skin colour. This was carried out by harvesting five plants per cultivar, each one filling a box from which were removed two clusters for evaluation. Brix determination was by means of an *Atago* refractometer model *Palette–PR 100*: firmness was measured with a *Chatillon* penetrometer with a tooth-shaped point and measurements were recorded in Newton (N), equivalent to (m · kg · s$^{-2}$). Skin colour was measured with a Minolta CR-200 colorimeter and calibrated according to the L, a, b scale of Hunter where L is a measure of the level of light or dark (from L=0 for an absence of reflection, or black, to L=100 for a perfect reflecting diffuser). For the a and b scales, a negative a is green and a positive one is red. A positive b is yellow and a negative one is blue. Postharvest quality was measured in the postharvest laboratory of the university of Costa Rica on fruits produced during the second production cycle.

The mean values were calculated for each plot and production cycle. The data were then subjected to an ANOVA by means of PROC MIXED of SAS (1999-2001), assuming that the interactions cultivar*block and production cycle*block are random.
Results

In both ‘Yangambi km 5’ and ‘Dátil’, pseudostem height and girth increased during the second production cycle. There were no differences between cultivars in the number of leaves at flowering and harvest, or in the height and number of foliage leaves of the sucker between the first and second production cycles (Table 1).

In ‘Yangambi km 5’ pseudostem height and girth were lower than in ‘Dátil’ during the first production cycle only. There were no differences between cultivars in the numbers of leaves at flowering and at harvest, or in the height of the sucker and its number of foliage leaves during the first or second production cycles. There were no differences in the number of days from planting to first flowering or from the first to the second flowering (Table 1).

During the second production cycle, ‘Yangambi km 5’ showed an increase in the weight of the bunch and rachis, and in the number of hands and fruits. There was a reduction in thickness of the central fruit of the outer row of the second, fourth and sixth hands. There were no differences between the production cycles in the length of the same fruit from the same hands (Table 2).

In ‘Dátil’, the weight of the bunch and rachis and the number of hands were similar over the two production cycles, but there were more fruits in the second production cycle. With the exception of the outer length of the middle fruit of the sixth hand, there was a reduction in the thickness and length in the fruits of the evaluated hands (Table 2).

In the first production cycle, neither cultivar differed in rachis weight and number of hands, but ‘Yangambi km 5’ presented higher values of these parameters than ‘Dátil’ in the second production cycle. ‘Yangambi km 5’ had thinner and longer fruits than ‘Dátil’ over both production cycles (Table 2).

The flesh of ‘Yangambi km 5’ was less sweet and firmer than the one of ‘Dátil’ and the yellow colour of the skin was not as intense as in ‘Dátil’ (Table 3).

‘Yangambi km 5’ had 2.1 and 2.6 times more total and functional roots respectively than ‘Dátil’, and 1.2 times functional roots in percentage terms (Table 4). Nematodes (Radopholus spp., Helicotylenchus spp., Meloydogine spp., Pratylenchus spp.) were

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Table 1. Mean values of various agronomic parameters in ‘Yangambi km 5’ (AAA) and ‘Dátil’ (AA) over two production cycles (n=6 plots).

<table>
<thead>
<tr>
<th>Production cycle</th>
<th>Pseudostem height (m)</th>
<th>Pseudostem girth (cm)</th>
<th>Numbers of leaves at flowering</th>
<th>Numbers of leaves at harvest</th>
<th>Number of days from planting to first flowering</th>
<th>Number of days from first to second flowering</th>
<th>Sucker height (m)</th>
<th>Sucker number of foliage leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yangambi km 5</td>
<td>1</td>
<td>2.2</td>
<td>11.8</td>
<td>12.0</td>
<td>8.3</td>
<td>280</td>
<td>2.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Yangambi km 5</td>
<td>2</td>
<td>3.7</td>
<td>15.3</td>
<td>12.4</td>
<td>8.3</td>
<td>261</td>
<td>2.4</td>
<td>9.1</td>
</tr>
<tr>
<td>Dátil</td>
<td>1</td>
<td>3.2</td>
<td>12.8</td>
<td>11.9</td>
<td>8.0</td>
<td>307</td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Dátil</td>
<td>2</td>
<td>3.9</td>
<td>15.5</td>
<td>11.8</td>
<td>7.8</td>
<td>255</td>
<td>2.3</td>
<td>8.6</td>
</tr>
<tr>
<td>Standard error</td>
<td></td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>14.9</td>
<td>12.8</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Pr&gt; F Yangambi km 5 vs Dátil</td>
<td>1 vs 2</td>
<td>0.0002</td>
<td>0.0005</td>
<td>0.2273</td>
<td>0.1969</td>
<td>0.1952</td>
<td>0.0717</td>
<td></td>
</tr>
<tr>
<td>Pr&gt; F Dátil</td>
<td>1 vs 2</td>
<td>0.0051</td>
<td>0.0012</td>
<td>0.7518</td>
<td>0.6590</td>
<td>0.3119</td>
<td>0.2656</td>
<td></td>
</tr>
<tr>
<td>Pr&gt; F Yangambi km 5 vs Dátil</td>
<td>1</td>
<td>0.0012</td>
<td>0.0457</td>
<td>0.8265</td>
<td>0.3041</td>
<td>0.2404</td>
<td>0.7919</td>
<td>0.9174</td>
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<tr>
<td>Pr&gt; F Yangambi km 5 vs Dátil</td>
<td>2</td>
<td>0.2984</td>
<td>0.5218</td>
<td>0.1168</td>
<td>0.1978</td>
<td>0.7706</td>
<td>0.5062</td>
<td>0.4139</td>
</tr>
</tbody>
</table>

*Standard error of mean.

Table 2. Mean values of various production parameters in ‘Yangambi km 5’ (AAA) and ‘Dátil’ (AA) over two production cycles (n=6 plots).

<table>
<thead>
<tr>
<th>Production cycle</th>
<th>Bunch weight (kg)</th>
<th>Rachis weight (kg)</th>
<th>Number of hands</th>
<th>Number of fruits per bunch</th>
<th>Diameter of middle fruit of outer row of nth hand (mm)</th>
<th>Length of middle fruit of outer row of n+1th hand (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>Fourth</td>
<td>Sixth</td>
<td>Second</td>
<td>Fourth</td>
<td>Sixth</td>
<td></td>
</tr>
<tr>
<td>Yangambi km 5</td>
<td>1</td>
<td>8.1</td>
<td>0.9</td>
<td>5.9</td>
<td>95</td>
<td>30.1</td>
</tr>
<tr>
<td>Yangambi km 5</td>
<td>2</td>
<td>11.7</td>
<td>1.2</td>
<td>8.3</td>
<td>165</td>
<td>28.0</td>
</tr>
<tr>
<td>Dátil</td>
<td>1</td>
<td>11.0</td>
<td>1.0</td>
<td>6.5</td>
<td>122</td>
<td>33.6</td>
</tr>
<tr>
<td>Dátil</td>
<td>2</td>
<td>9.7</td>
<td>0.9</td>
<td>7.2</td>
<td>150</td>
<td>30.4</td>
</tr>
<tr>
<td>Standard error*</td>
<td></td>
<td>0.49</td>
<td>0.06</td>
<td>0.26</td>
<td>7.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Pr&gt; F Yangambi km 5 vs Dátil</td>
<td>1 vs 2</td>
<td>0.0062</td>
<td>0.0188</td>
<td>0.0014</td>
<td>0.0030</td>
<td>0.0033</td>
</tr>
<tr>
<td>Pr&gt; F Dátil</td>
<td>1 vs 2</td>
<td>0.1518</td>
<td>0.5393</td>
<td>0.0717</td>
<td>0.0620</td>
<td>0.0008</td>
</tr>
<tr>
<td>Pr&gt; F Yangambi km 5 vs Dátil</td>
<td>1</td>
<td>0.0139</td>
<td>0.3888</td>
<td>0.2064</td>
<td>0.0678</td>
<td>0.0006</td>
</tr>
<tr>
<td>Pr&gt; F Yangambi km 5 vs Dátil</td>
<td>2</td>
<td>0.0459</td>
<td>0.0243</td>
<td>0.0438</td>
<td>0.2455</td>
<td>0.0021</td>
</tr>
</tbody>
</table>

*Standard error of mean.
not found in the roots of either cultivar (Table 4). Weevils (Cosmoptilus sordidus) were not found in the corms of ‘Yangambi km 5’, whereas a small quantity of weevils was found in the ones of ‘Dátil’ (Table 5).

**Discussion**

According to Shepherd et al. (1986), triploid cultivars, particularly those in commercial plantations, are generally superior to diploids in terms of vigour, productivity and acceptability. In the present work, whilst ‘Dátil’ was more vigorous and productive than ‘Yangambi km 5’ during the first production cycle, the data from the second production cycle confirm this observation, with the exception of the variables related to fruit quality which, in ‘Yangambi km 5’, were inferior to the ones observed in ‘Dátil’.

The increases in plant height, pseudostem girth and numbers of hands per bunch observed in both cultivars during the second production cycle, translated into a higher bunch weight only in ‘Yangambi km 5’. This was mainly due to an increase in the number of fruits per bunch, which were thinner and of similar length to those in the first production cycle. This was different from ‘Dátil’, whose smaller fruits in the second production cycle were not more numerous. Given that both cultivars had a similar and adequate number of leaves at flowering and harvest, the differences in productivity were not due to black leaf streak.

Paradoxically, the increased vigour of ‘Dátil’ did not translate into a higher increased productivity, suggesting that in this cultivar there is a similar decline in productivity to that in plantain type False horn (AAAB). In these materials (Perea 2003, Pantoja et al. 1995, Swennen et al. 1984), the decline in productivity increases with the number of production cycles. Perea (2003) and Pantoja et al. (1995) report not knowing the cause of such a decline and suggest that the plant could be weakened by diseases, weevils and nematodes and by environmental factors or agronomic management. Nevertheless, given the favourable conditions, whether agronomic or climatic and related to crop sanitation under which the present work was conducted, and as reflected in increased plant vigour over the production cycles, it is very unlikely that the decline in ‘Dátil’ was associated with the previously mentioned factors. It could be specific to the genotype, probably as a result of a reduced root system.

The root system, together with the increase in height observed in the second production cycle, increased the susceptibility of the plants to toppling. Given that the land had not been cultivated for some time, it is likely the populations of phytoparasitic nematodes affecting Musa were reduced, a situation that could have been responsible for their absence in the roots of ‘Dátil’, a cultivar considered susceptible to R. similis (Stoffelen et al. 1999), Meloidogyne incognita, Meloidogyne javanica (Stoffelen et al. 1999, De Waele and Davide 1998) and Pratylenchus coffeae (Stoffelen et al. 1999).

However, this latter would not be important in ‘Yangambi km 5’, a cultivar considered to be resistant to R. similis and P. coffeae (Viaene et al. 2000, Sarah et al. 1996) nor, in the case of weevil, to which both are considered to be resistant (Gold et al. 2002, Hasyim and Gold 1998).

The better fruit quality of ‘Dátil’ observed in this study, as well as the greater interest in exporting it as fresh fruit (Buitrago et al. 1994, Jamaluddin 1990, Daniels 1986, Contreras 1982), suggests that there is a need to further evaluate the intensive production strategies such as the one developed for plantains (Belalcázar 1991, Vargas 1994).

---

**Table 3. Mean values for Brix, firmness of flesh and skin colour using the Hunter color in ‘Yangambi km 5’ (AAA) and ‘Dátil’ (AA) (n=10 clusters).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>‘Yangambi km 5’</th>
<th>‘Dátil’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brix (%)</td>
<td>18.2 ± 0.5</td>
<td>24.5 ± 0.5</td>
</tr>
<tr>
<td>Firmness (N)</td>
<td>4.4 ± 0.4</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>L</td>
<td>79.6 ± 0.5</td>
<td>82.4 ± 0.7</td>
</tr>
<tr>
<td>a</td>
<td>-5.1 ± 0.5</td>
<td>-9.1 ± 0.6</td>
</tr>
<tr>
<td>b</td>
<td>49.8 ± 1.3</td>
<td>55.4 ± 1.1</td>
</tr>
</tbody>
</table>

**Table 4. Root content (mean ± standard deviation) in ‘Yangambi km 5’ (AAA) and ‘Dátil’ (AA) (n=15 plants).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>‘Yangambi km 5’</th>
<th>‘Dátil’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total roots (g)</td>
<td>119 ± 27</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>Functional roots (g)</td>
<td>112 ± 28</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Functional roots (%</td>
<td>94 ± 2</td>
<td>76 ± 3</td>
</tr>
</tbody>
</table>

**Table 5. Estimated damage (mean ± standard deviation) by weevil larvae in ‘Yangambi km 5’ (AAA) and ‘Dátil’ (AA) (n=48 plants in each production cycle for ‘Yangambi km 5’, and n=41 and 31 plants in first and second production cycles respectively for ‘Dátil’).**

<table>
<thead>
<tr>
<th>Production cycle</th>
<th>Infestation (%)</th>
<th>Degree of rot</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Yangambi km 5’</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>‘Dátil’</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>‘Yangambi km 5’</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>‘Dátil’</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Standard error of mean.
This should include consideration of high plantation densities, replanting the field after each production cycle and spreading out planting over time, practices which, for plantain, have been shown to be a more profitable and reliable management option that traditional system.

Acknowledgements

The authors thank Ing. Marco Vinicio Sáenz MSc., Laboratorio Poscosecha de la Universidad de Costa Rica and Ing. Mauricio Serrano for their collaboration.

References


Genetic improvement of banana and plantain in the various programmes operating around the world is based on crosses between commercial triploids and improved diploids, with the objective of developing higher yielding cultivars that are more resistant to the main diseases (Sigatoka disease caused by *Mycosphaerella musicola*, black leaf streak caused by *Mycosphaerella fijiensis*, fusarium wilt caused by *Fusarium oxysporum* f.sp. cubense), and pests (nematodes and weevils).

In the last years, various research groups throughout the world have made concerted efforts to increase genetic variability, which is of major importance for the selection of clones with higher productivity and resistance to the main diseases (López 1989).

The current study was to determine the combinations of parents that provide the best opportunity for producing new hybrids possessing the desirable agronomic characters.

**Materials and methods**

The present work was carried out at the *Instituto de Investigaciones en Viandas Tropicales* (INIVIT), Santo Domingo, Cuba from January 1995 to December 2003 using conventional hybridization methods. Table 1 describes the genomic group of the parents used. The *Musa balbisiana* used is a wild diploid from the INIVIT collection, originally from Vietnam, which is very resistant to toppling and the main banana diseases. A total of 3500 crosses representing 17 combinations were obtained.

Selected parents were planted in the field in alternate furrows at a distance of 3.6 m x 2 m. The soil had brown mottles and carbonates (Hernández 1995). Management was according to technical instructions for the cultivation of plantain (MINAGRI 1994).

Ten months after planting, at the peak of flowering, male flowers with abundant pollen sacs were selected and spread vigorously over the stigma of female flowers that had opened in the morning. The process was repeated with flowers that were in the process of opening (Silva et al. 1997).

The pollinated bunches thus obtained were harvested when they reached maturity. They were transferred to a maturation room, grouped according to their crossing scheme, where they remained until complete maturation. The fingers were cut longitudinally to remove the seeds. The seeds were sieved (3 mm mesh) under a steady flow of running water in order to remove the flesh, and transferred to flasks of distilled water to start disinfection and dissection.

The regenerated plants were established in polythene bags, with a substrate composed of 50% soil and 50% organic matter from a partially decomposed by-product of sugar cane, and transferred to the field when ready.

The best combinations were determined on the basis of the numbers of seeds obtained and the value of the progenies obtained.

**Results and discussion**

In the first crosses, *Musa balbisiana* (BB) or *Musa acuminata* ssp. *malaccensis* (AA), was crossed with ‘Highgate’ or ‘Hembra ¾’ as the female parent. The progenies showed resistance to Fusarium wilt and black leaf streak but inherited the defects of the male parent, such as asymmetrical bunches, short fingers and high seed content. These progenies were discarded as having no commercial value. Table 2 presents data on the numbers of good and bad seeds.

**Crosses between SH-3436-L9 and *Musa balbisiana* (BB)**

No satisfactory results were obtained in the process of improving AAA and AAAA cultivars.

---

**Table 1. Name and genomic group of parents used.**

<table>
<thead>
<tr>
<th>Male parents</th>
<th>Female parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paka (AA)</td>
<td>Highgate (AAA)</td>
</tr>
<tr>
<td>Pisang jari buaya (AA)</td>
<td>Hembra ¾ (AAB)</td>
</tr>
<tr>
<td>Calcutta 4 (AA)</td>
<td>Pelipita (ABB)</td>
</tr>
<tr>
<td>SH-3142 (AA)</td>
<td>Saba (ABB)</td>
</tr>
<tr>
<td>SH-3362 (AA)</td>
<td>Saba somadone (ABB)</td>
</tr>
<tr>
<td><em>Musa balbisiana</em> (BB)</td>
<td>SH-3436-L9 (AAAA)</td>
</tr>
</tbody>
</table>
The tetraploid SH-3436 (AAAA) is a product of the improvement programme of the Fundación Hondureña de Investigación Agrícola (FHIA). Starting with this hybrid, INIVIT obtained through tissue culture the somaclonal variant SH-3436-L9. This clone originates from ‘Highgate’, as do most of the tetraploids produced by FHIA. When used as a female parent and pollinated by a diploid, this semi-dwarf mutant of ‘Gros Michel’ (AAA) produces non-reduced gametes to generate tetraploids.

When SH-3436-L9 was crossed with *Musa balbisiana*, not only was the number of seeds small (1 seed/bunch), but the seeds also had abnormal endosperm and embryos and did not germinate. The use of SH-3436-L9 as a male parent was not promising. None of the progenies survived the selection processes or showed any agronomic value.

Using wild *Musa acuminate*, Larter (1947) obtained tetraploids that were reasonably satisfactory from a commercial point of view, but that had inherited the non-parthenocarpic characteristics of the male parent. Since seeds are an undesirable character, the tetraploids should be female sterile.

**Crosses between SH-3436-L9 and ‘Pisang jari buaya’ or ‘Paka’**

Crossing SH-3436-L9 with ‘Pisang jari buaya’ and ‘Paka’ produced only a few seeds that did not germinate and had abnormal embryos and endosperms, for reasons that are not understood.

**Crosses between ‘Highgate’ and SH-3142 or SH-3362**

Crosses between ‘Highgate’ and SH-3142 and SH-3362 produced very few seeds (1 to 2 seeds per pollinated bunch). Progenies from these crosses died at the greenhouse stage.

**Crosses between ‘Highgate’ and ‘Calcutta 4’ or ‘Paka’**

The use of ‘Calcutta 4’ produced progenies with seedless but small fruits and dwarf plants that were therefore discarded. No seeds were produced when ‘Highgate’ was crossed with ‘Paka’.

**Crosses between ‘Hembra ¾’ and SH-3142, SH-3362 or ‘Calcutta 4’**

When ‘Hembra ¾’ was crossed with SH-3142 or SH-3362, one to two seeds per bunch were obtained, as when it was pollinated with ‘Calcutta 4’. The seeds from these crosses were not viable.

**Crosses between ‘Pelipita’ and ‘Pisang jari buaya’**

The genetic improvement of cooking bananas (ABB) involved crossing the cultivar ‘Pelipita’, which is resistant to black leaf streak, *Fusarium* wilt and nematodes, with ‘Pisang jari buaya’, which is highly resistant to nematodes. The result was a tetraploid progeny of considerable height (more than 3.5 m) with small bunches and asymmetrical fingers of unacceptable quality. Flowering took place 15 to 18 months after planting. The progeny from this cross was discarded for these reasons. On the other hand, the descendents had inherited the resistance to nematodes.

The primary tetraploids obtained from these crosses could be used as female parents in crosses with improved diploids to obtain secondary triploids and to increase genetic diversity.

**Crosses between ‘Pelipita’ and ‘Calcutta 4’**

When ‘Pelipita’ was crossed with ‘Calcutta 4’, the progeny had thick leaves and a dwarf habit, and did not survive in the greenhouse. The plants appeared to be heptaploid although this was not verified.

**Crosses between ‘Saba’ or a somaclone of ‘Saba’ and ‘Pisang jari buaya’**

Crossing a ‘Saba’ somaclone with ‘Pisang jari buaya’ yielded a small number of seeds that had reduced endosperms and abnormal embryos, and did not germinate.

---

**Table 2. Mean number of seeds per bunch (n = 20 pollinated bunches) obtained from various crosses.**

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>Mean number of seeds per pollinated bunch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Good</td>
</tr>
<tr>
<td>SH-3436-L9 (AAAA)</td>
<td><em>Musa balbisiana</em> (BB)</td>
<td>0.0</td>
</tr>
<tr>
<td>SH-3436-L9 (AAAA)</td>
<td>‘Pisang jari buaya’ (AA)</td>
<td>0.0</td>
</tr>
<tr>
<td>SH-3436-L9 (AAAA)</td>
<td>‘Paka’ (AA)</td>
<td>0.0</td>
</tr>
<tr>
<td>‘Highgate’ (AAA)</td>
<td>SH-3142 (AA)</td>
<td>2.5</td>
</tr>
<tr>
<td>‘Highgate’ (AAA)</td>
<td>SH-3362 (AA)</td>
<td>1.0</td>
</tr>
<tr>
<td>‘Highgate’ (AAA)</td>
<td>‘Calcutta 4’ (AA)</td>
<td>5.0</td>
</tr>
<tr>
<td>‘Highgate’ (AAA)</td>
<td>‘Paka’ (AA)</td>
<td>0.0</td>
</tr>
<tr>
<td>‘Hembra ¾’ (AAB)</td>
<td>SH-3142 (AA)</td>
<td>0.0</td>
</tr>
<tr>
<td>‘Hembra ¾’ (AAB)</td>
<td>SH-3362 (AA)</td>
<td>0.0</td>
</tr>
<tr>
<td>‘Hembra ¾’ (AAB)</td>
<td>‘Calcutta 4’ (AA)</td>
<td>0.0</td>
</tr>
<tr>
<td>‘Pelipita’ (ABB)</td>
<td>‘Pisang jari buaya’ (AA)</td>
<td>14.5</td>
</tr>
<tr>
<td>‘Pelipita’ (ABB)</td>
<td>‘Calcutta 4’ (AA)</td>
<td>6.4</td>
</tr>
<tr>
<td>‘Saba’ (ABB)</td>
<td>‘Pisang jari buaya’ (AA)</td>
<td>3.7</td>
</tr>
<tr>
<td>‘Saba somaclone’ (ABB)</td>
<td>‘Pisang jari buaya’ (AA)</td>
<td>2.9</td>
</tr>
</tbody>
</table>
Crosses between a somaclone of ‘Saba’ and SH-3362 or SH-3142

When a ‘Saba’ somaclone was crossed with SH-3362, which is highly resistant to black leaf streak, 14.6 seeds per pollinated bunch were obtained. The seeds had normal endosperms and embryos. The seeds were opened in the laboratory and produced 70 plants, of which 12 survived in the greenhouse, when they were put on culture medium. The remaining plants were discarded as having undesirable characteristics such as thick leaves and a dwarf habit. The plants that were planted in the field were tetraploids that had acceptable bunch characters inherited from the male parent (pendulous with 8 or 9 hands, 90 to 100 fingers per bunch). At the present time, the plantings in the field are in their second production cycle undergoing evaluation for their commercial potential and their response to different pests and diseases.

When the ‘Saba’ somaclone was crossed with SH-3142, 350 seeds were obtained, of which only 100 proved viable; the remainder were eliminated as having dry and abnormal embryos. Mean seed numbers per bunch varied between 14 and 20 seeds.

References


New methodology for the establishment of cell suspensions of ‘Grande naine’ (AAA)


Most research groups initiate cell suspensions of the cultivar ‘Grande naine’ (AAA) from immature male flowers. However, the formation of calli with embryogenic structures takes five to six months and the success rate is between 3% and 10%. Furthermore, after obtaining these structures, the proportion of established embryogenic cell suspensions do not exceed 30% in the two months needed to become homogenous. The objectives of this work were to establish cell suspensions by cultivating immature male flowers of the cultivar ‘Grande naine’ directly on liquid culture medium, and to achieve the formation of somatic embryos from these suspensions and regenerate the plants.

Materials and methods

Immature male flowers of the banana cultivar ‘Grande naine’ were used as vegetative material. Immature male flowers were extracted according to the methodology described in Escalant et al. (1994).

Effect of the immature male flower’s position on cell suspension initiation

Only male flowers in the 5th to 15th position were taken. Ten immature male flowers from the same position were taken from 10 male buds and transferred to 50 ml Erlenmeyer flasks containing 5.0 ml of a MA1 liquid cultivation medium prepared with salts and MS vitamins (Murashige and Skoog 1962) supplemented with 4.09 µM biotin, 5.7 µM indole-3-acetic acid (IAA), 18.1 µM 2,4-dichlorophenoxyacetic acid (2,4-D), 5.37 µM naphleneacetic acid (NAA) and 87.6 mM saccharose. The pH was adjusted to 5.7 before sterilization with 1 N NaOH and 1 N HCl (Escalant et al. 1994). Erlenmeyer flasks were placed on an orbital shaker model INFORS (HT), in constant dark, at a speed of 90 rpm and a temperature of 27±0.2°C. Half of the culture medium was replaced every 15 days until the appearance of cells; from then on the culture medium was changed weekly.

After 45 days, the presence or absence of yellow globular structures on the surface of the immature male flowers was evaluated. After 70 days of culture, the quality of the cell
suspensions was evaluated by determining cell viability with fluorescein diacetate (FDA) and cell counts were made in a Neubauer chamber. Similar evaluations were made in all experiments as required.

**Effect of culture medium on cell suspension initiation**

Immature male flowers from the 7th to the 11th position, ten per position, were collected and transferred to 50 ml Erlenmeyer flasks containing 5 ml of culture medium. The MA1 liquid culture medium was compared to the MA2 medium for the establishment and multiplication of cell suspensions described in Côte *et al.* (1996). The latter contained salts and MS vitamins supplemented with 4.09 µM biotin, 4.5 µM 2,4-D, 680 µM L-glutamine, 100 mg/L malt extract and 130 mM saccharose. The pH was adjusted to 5.3 before sterilization by autoclaving. The culture conditions and variables in evaluation were the same as in the previous experiment.

**Multiplication of embryogenic cell suspensions and formation of somatic embryos**

After 100 days from the start of the experiment, cell suspensions were sieved on a metal mesh with a pore size of 500 µm. The filtrates were the cell suspensions which were transferred to a MA2 culture medium for maintenance or multiplication.

Two hundred µl of settled cells from three different cell lines, all obtained by using our methodology, were plated on Petri dishes 9 cm in diameter and containing the MA3 culture medium described in Côte *et al.* (1996): salts (Schenk and Hildebrandt 1972), MS vitamins, 680 µM L-glutamine, 2 mM L-proline, 1.1 µM NAA, 0.7 µM adenine N\(^6\)-(2-isopentenol), 0.5 µM kinetin, 29 mM lactose, 0.2 µM zeatin, 100 mg/L malt extract, 130 mM saccharose and solidified with 3.0 g/L Gelrite. The pH was adjusted to 5.3 before sterilization.

Petri dishes were sealed with Parafilm® and placed in total darkness at a temperature of 27±2°C. Visual observations were made daily after the fifteenth day of culture to evaluate the appearance of the first somatic embryos. Counts of somatic embryos were made after 45 days of culture and no subculturing was done.

The somatic embryos were transferred to the culture medium described in Gómez *et al.* (2000) for maturing. The culture medium was prepared with salts and MS vitamins supplemented with 4.09 µM biotin, 2.22 µM 6-benzylaminopurine, 1.1 µM IAA, 130 mM saccharose and 2.0 g/L Gelrite, with the pH adjusted to 5.8 before sterilization. Culture conditions were total darkness at 27±2°C and the embryos remained in the culture medium for 30 days.

Mature somatic embryos were then placed on the germination culture medium (M5) described in Gómez *et al.* (2000) and containing salts and MS vitamins supplemented with 4.1 µM IAA, 0.2 µM 6-benzylaminopurine, 0.01 mg/L Biobras-6, 87 mM saccharose, solidified with 2 g/L Gelrite, and adjusted to pH 5.8 before autoclaving. The culture flasks had a capacity of 250 ml and contained 30 ml of culture medium. Flasks were placed in growth chambers with sunlight and exposed to a flux of photosynthetic photons of 50 - 62.5 µmol m\(^{-2}\) s\(^{-1}\) and a temperature of 27±2°C.

Data were analysed by means of the statistical packages SPSS ver. 9.0 and StatGraphics Plus ver. 4.1.

**Results**

**Effect of the immature male flower’s position**

Immature male flowers cultivated directly on liquid culture medium responded after two weeks in culture. Phenol formation was observed in the cortex zone followed by enlargement of the explants. The biggest response occurred with flowers from the 8th to 15th position, with no significant differences between them (Table 1). Flowers from positions 5 and 6 did not respond satisfactorily, with the majority observed to have total tissue necrosis. After five weeks of culture, yellow globular structures appeared on the surface of the explants, which rapidly separated from the latter (Figure 1A). There were significant statistical differences between the different positions with regards to the quantity of explants that formed such types of structure (Table 1). Immature male flowers from positions 7 to 11 had the largest quantity of explants with such structures (70‐90%), with no significant differences between positions. Explants from positions 12 to 15
had a disorganised growth but formed very few of these structures. After 10 weeks of culture, turbidity was observed at the bottom of all Erlenmeyer flasks. Observation under the optical microscope at 200x magnification revealed the presence of small spherical embryogenic cells with dense cytoplasm, starch granules and various aggregates of embryos. Cell vacuoles were also observed.

Counts of cell aggregates, single cells and vacuolate cells, showed that flowers from the 8th position yielded the largest quantity of embryogenic cell aggregates (3.0x10⁴) and the least quantity of vacuolate cells (1.4x10⁳) (Table 1). Positions 5 and 6 generally produced very few cells, whereas positions 11 to 15 produced large quantities of single cells and vacuolate cells, which is not desirable.

**Influence of culture medium**

There were significant differences, for all variables evaluated, between treatments looking at the interaction between the position of the immature male flowers and the type of culture medium (Table 2). The best responses with regards to the formation of yellow globular structures were obtained with immature male flowers from positions 8, 9, 10 and 11 in MA1 culture medium, and position 10 in MA2 culture medium, with 85% to 88% of the explants producing yellow globular structures.

After 10 weeks of culture, cells were observed in all the treatments. The treatment with the best results was position 8 in culture medium MA1, with the biggest quantity of embryogenic cells (3.1x10⁴) and the lowest quantity of vacuolate cells (1.3x10⁵) per millilitre of culture medium

### Table 1. Effect of culture medium and ranking of immature male flowers of ‘Grande naine’ (AAA) after 5 weeks culture and composition of cell suspensions after 10 weeks of culture (n=10).

<table>
<thead>
<tr>
<th>Position of male flower</th>
<th>% of explants enlarged</th>
<th>% of explants with globular structures</th>
<th>Number (x10⁵) of single cells per ml of culture medium</th>
<th>Number (x10⁵) of cell aggregates per ml of culture medium</th>
<th>Number (x10⁵) of vacuolate cells per ml of culture medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10 c**</td>
<td>0.0 b*</td>
<td>1.8 ± 1.2 d**</td>
<td>0.1 ± 0.1 g**</td>
<td>5.3 ± 5.7 f**</td>
</tr>
<tr>
<td>6</td>
<td>10 c</td>
<td>7.5 b</td>
<td>2.2 h</td>
<td>0.1 g</td>
<td>4.5 g</td>
</tr>
<tr>
<td>7</td>
<td>80 b</td>
<td>72.5 a</td>
<td>8.6 e</td>
<td>2.4 b</td>
<td>2.5 i</td>
</tr>
<tr>
<td>8</td>
<td>90 ab</td>
<td>90.0 a</td>
<td>8.9 e</td>
<td>3.0 h</td>
<td>1.4 j</td>
</tr>
<tr>
<td>9</td>
<td>90 ab</td>
<td>90.0 a</td>
<td>1.2 c</td>
<td>1.7 c</td>
<td>2.1 h</td>
</tr>
<tr>
<td>10</td>
<td>100 a</td>
<td>70.0 a</td>
<td>15.2 b</td>
<td>1.8 c</td>
<td>5.4 f</td>
</tr>
<tr>
<td>11</td>
<td>100 a</td>
<td>87.8 a</td>
<td>18.3 a</td>
<td>1.3 d</td>
<td>7.4 e</td>
</tr>
<tr>
<td>12</td>
<td>100 a</td>
<td>17.5 b</td>
<td>14.9 b</td>
<td>1.2 d</td>
<td>11.3 d</td>
</tr>
<tr>
<td>13</td>
<td>100 a</td>
<td>2.5 b</td>
<td>11.0 d</td>
<td>0.5 e</td>
<td>17.2 c</td>
</tr>
<tr>
<td>14</td>
<td>100 a</td>
<td>2.5 b</td>
<td>6.6 f</td>
<td>0.5 e</td>
<td>24.5 b</td>
</tr>
<tr>
<td>15</td>
<td>100 a</td>
<td>2.5 b</td>
<td>5.6 g</td>
<td>0.3 f</td>
<td>28.4 a</td>
</tr>
<tr>
<td>SE</td>
<td>±5.2</td>
<td>±4.2</td>
<td>±5.4</td>
<td>±1.0</td>
<td>±8.0</td>
</tr>
</tbody>
</table>

*Values followed by different letters in the same column are significantly different at p< 0.05 according to Tukey's test.

**Values followed by different letters in the same column are significantly different at p< 0.05 according to Dunett’s C test.

### Table 2. Effect of culture medium and ranking of immature male flowers of ‘Grande naine’ (AAA) after 5 weeks culture and composition of cell suspensions after 10 weeks culture (n=10).

<table>
<thead>
<tr>
<th>Position of male flower</th>
<th>Culture medium</th>
<th>% of explants enlarged</th>
<th>% of explants with globular structures</th>
<th>Number (x10⁵) of single cells per ml of culture medium</th>
<th>Number (x10⁵) of cell aggregates per ml of culture medium</th>
<th>Number (x10⁵) of vacuolate cells per ml of culture medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>MA1</td>
<td>92.5 g*</td>
<td>77.5 cd*</td>
<td>8.6 ± 1.2 d**</td>
<td>2.6 ± 1.6 d**</td>
<td>2.5 ± 0.6 b**</td>
</tr>
<tr>
<td>8</td>
<td>MA1</td>
<td>92.5 a</td>
<td>85.0 ab</td>
<td>8.9 ± 1.5 d</td>
<td>1.3 ± 0.7 e</td>
<td>3.1 ± 1.2 a</td>
</tr>
<tr>
<td>9</td>
<td>MA1</td>
<td>95.0 a</td>
<td>87.5 a</td>
<td>12.1 ± 2.1 c</td>
<td>2.0 ± 0.5 c</td>
<td>2.0 ± 1.2 c</td>
</tr>
<tr>
<td>10</td>
<td>MA1</td>
<td>95.0 a</td>
<td>85.0 a</td>
<td>15.2 ± 2.1 b</td>
<td>5.7 ± 2.9 b</td>
<td>1.9 ± 0.5 c</td>
</tr>
<tr>
<td>11</td>
<td>MA1</td>
<td>97.5 a</td>
<td>87.5 a</td>
<td>18.2 ± 1.7 a</td>
<td>7.7 ± 1.1 a</td>
<td>1.3 ± 0.5 e</td>
</tr>
<tr>
<td>7</td>
<td>MA2</td>
<td>25.0 b</td>
<td>40.0 e</td>
<td>1.9 ± 0.7 h</td>
<td>3.9 ± 1.3 c</td>
<td>0.8 ± 0.4 fg</td>
</tr>
<tr>
<td>8</td>
<td>MA2</td>
<td>47.5 b</td>
<td>65.0 d</td>
<td>5.8 ± 1.4 f</td>
<td>2.3 ± 0.9 d</td>
<td>1.6 ± 0.4 d</td>
</tr>
<tr>
<td>9</td>
<td>MA2</td>
<td>85.0 a</td>
<td>80.0 bc</td>
<td>6.2 ± 1.7 f</td>
<td>2.3 ± 0.5 d</td>
<td>0.9 ± 0.4 f</td>
</tr>
<tr>
<td>10</td>
<td>MA2</td>
<td>97.5 a</td>
<td>85.0 ab</td>
<td>5.9 ± 1.3 f</td>
<td>6.0 ± 2.2 b</td>
<td>0.6 ± 0.3 g</td>
</tr>
<tr>
<td>11</td>
<td>MA2</td>
<td>100 a</td>
<td>37.5 e</td>
<td>2.8 ± 1.3 g</td>
<td>7.8 ± 1.9 a</td>
<td>0.4 ± 0.2 h</td>
</tr>
<tr>
<td>SE</td>
<td>±5.2</td>
<td>±9.2</td>
<td>±5.2</td>
<td>±2.32</td>
<td>±0.9</td>
<td></td>
</tr>
</tbody>
</table>

*Values followed by different letters in the same column are significantly different at p< 0.05 according to Tukey’s test.

**Values followed by different letters in the same column are significantly different at p< 0.05 according to Dunett’s C test.
These results support those obtained in the previous experiment where position 8 gave the best results in terms of the establishment of embryogenic cell suspensions. No references were found on the use of immature male flowers directly on a liquid culture medium.

These results may be due to the stage of differentiation of immature male flowers and to the composition of the MA1 culture medium. This culture medium has a higher concentration of auxins than the MA2 medium, in addition to having a mixture of them (2,4-D, IAA and NAA), whereas the culture medium MA2 only has one auxin, 2,4-D, at a lower concentration (1 mg/L).

According to Parrot (1993), the type, physiological state and degree of differentiation and polarization of the tissues used as initial explants are some of the factors that favour or impede the formation of calli with embryogenic structures. In an experiment using ‘Grande naine’, immature male flowers from positions 7 to 13, 74% of the calli formed embryogenic structures in a semisolid culture medium (Escalant et al. 1994). Noceda et al. (1999) studied the effect of various factors on callus production with embryogenic structures in ‘Grande naine’ and FHIA-18 (AAAB), and the factor that most affected the process was the time when male buds were harvested and the next most important factor was the position of the flower, with positions 5 to 8 giving the best results.

**Multiplication of embryogenic cell suspensions and formation of somatic embryos**

Given the results of the previous experiments, cell suspensions established from immature male flowers from the 8th position were inoculated directly onto MA1 culture medium in 50 ml Erlenmeyer flasks. The heterogeneous suspensions obtained were filtered to remove the globular structures and proembryos. After 20 weeks from the start of culture, and subculturing every 12 days, cell suspensions reached a high degree of homogeneity, mainly with aggregates of embryogenic cells characterized by small spherical embryogenic cells with dense cytoplasm, small vacuoles, starch granules and a well defined nucleus/cytoplasm.

The observation of cell suspensions verified that there had been changes in their composition with aggregates of embryogenic cells predominating and the quantity of single and vacuolate cells eventually decreasing to zero with each subculturing. These aggregates of embryogenic cells occupied 90% to 95% of the suspension and their size varied between 80 μm to 300 μm, resulting in a homogeneous cell suspension (Figure 1B).

The first embryos were observed 20 days after plating the three cell lines on MA3 culture medium. The number of embryos was counted after 45 days culture (Figure 1C). All cell lines had a good embryogenic response as determined by the quantity of somatic embryos formed. There were significant statistical differences (p<0.05 according to Duncan’s test, SE=102.3) between cell line 2, with the formation of a mean of 517 somatic embryos per 200 μl of plated settled cells, and cell lines 1 and 3, with the formation of 662 and 761 somatic embryos respectively. These differences could have resulted from differences in the embryogenic potential of the cell lines.

After 17 days of culture, the first somatic embryos started to germinate with the emergence of leaves and roots. Of the somatic embryos, 80.5% had completely germinated (Figure 1D) after 45 days of culture. It should be pointed out that no morphological changes were observed in the regenerated plants (Figure 1E).

**Discussion**

Our methodology made it possible to obtain homogeneous suspensions of embryogenic cells in 20 weeks (about five months) after the start of culture, in 70% of the explants inoculated. The methods proposed by Côte et al. (1996) and Grapin et al. (1996) take between four and seven months just for the formation of calli with embryogenic structures, and a further two months for the establishment of cell suspensions. Furthermore, it is necessary to take into account that the embryogenic response of male flowers cultured on semisolid medium was low. Escalant et al. (1994) reported that 0 to 7% of the explants had calli with embryogenic structures in five banana cultivars, including ‘Grande naine’. Daniels et al. (2002) reported a success rate of
6.5% with the hybrid FHIA-21 (AAAB). With ‘Grande naine’, the mean was 8% (Strosse et al. 2003). Khalil et al. (2002) obtained better results (58.8%) but with the cultivar ‘Brazilian dwarf’ (AAB). Moreover, the proportion of embryogenic cell suspensions established from ideal embryogenic calli was between 10% and 30% in ‘Grande naine’ (R. Domergue and colleagues, Cirad, unpublished results).

The number of somatic embryos formed in each ml cells varied between 7835 and 11 530, which demonstrates the quality of the cell suspensions obtained with our methodology. Values of 100 and 300 000 somatic embryos per ml have been reported in the literature (Côte et al. 1996, Grapin et al. 1996, Schoofs 1997, Daniels et al. 2002). The levels of germination of somatic embryos obtained after being in a maturation medium were on average 80.5%, a value higher than the 40.6% germination rate for the hybrid FHIA-21 using a maturation medium before germination.

Acknowledgements
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References

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*Email: borys_chong@yahoo.es
Environmental stress factors, such as drought, cold and salinity, impose a cost on plants trying to survive these severe conditions. Many genes have been implicated in the various physiological and biochemical responses induced in plants following a stress (Shinozaki and Yamaguchi-Shinozaki 1997, Shinozaki and Yamaguchi-Shinozaki 2000, Nuotio et al. 2001).

In bananas, growth ceases at about 10°C, irreversible damage happens at 0°C, and severe foliar damage is also observed at 37°C. However, little is known about the molecular, biochemical and physiological changes associated with disorders resulting from temperature extremes. In other species, damage has been mitigated by the exogenous application of plant growth regulators (González-Olmedo and Borroto 1987). The use of brassinosteroids to that end in horticulture is recent but so far it has given satisfactory results in reducing the effects of environmental stress (Núñez 1999, González-Olmedo et al. 2003).

The present work was carried out with the objective of controlling the effects of thermal stress on banana plantlets by the application of a brassinosteroid analogue during the acclimatization phase.

**Materials and methods**

Plantlets of FHIA-18 were produced by micropropagation in temporary immersion bioreactors by using a liquid MS medium (Murashige and Skoog 1962) supplemented with 6-benzylaminopurine (BAP) according to the protocol of Barrera et al. (2001).

At the end of the *in vitro* culture phase, plantlets were transferred to *ex vitro* conditions according to the protocol described in Anon. (2003). After four weeks in acclimatization houses, the plantlets were sprayed with a trihydroxilated spirotane analogue of brassinosteroid ($C_{27}O_{6}H_{42}$), (molecular mass = 462.606) at a rate of 2.2 ml per plant and a concentration of 0.1 mg/L. The control group was sprayed with water only. Two hours later, each group was subdivided into three and transferred for 72 h to different chambers with temperatures of 7°C, 27°C and 34°C. The variables measured were: rate of survival after 72 h in the chambers, after 10 days and at the end of acclimatization period; free proline content after 72 h in the chambers, as described in Bates et al. (1973); and at the end of the acclimatization period, number of necrotic leaves, plant fresh weight, number of roots, number of leaves and plant height.

**Effect of an analogue of brassinosteroid on FHIA-18 plantlets exposed to thermal stress**

J. L. González-Olmedo, A. Córdova, C. E. Aragón, D. Piña, M. Rivas and R. Rodríguez
stress. Statistical analysis was by parametric analysis (ANOVA, Tukey test, p<0.05) after checking the data for normal distribution (Kolmogorov-Smirnov) and homogeneity of variance (Bartlett).

Results and discussion

Table 1 presents the data on plantlet survival. Since the banana plantlets were already established and adapted to the temperatures outside the acclimatization chambers, changing the temperature did not noticeably affect their survival. Nevertheless, extreme temperatures resulted in some deaths, which occurred earlier in the treatments with cooler temperatures. No plantlets died in the treatments with the brassinosteroid.

Extreme temperatures increased the numbers of plants with stress symptoms, as measured by necrotic areas on the leaves, which were 38% and 97% at 34°C and 7°C respectively. In the plants treated with a brassinosteroid analogue the effects of the thermal stress were significantly reduced, with more than 53% and 5% of plants free of symptoms at 34°C and 7°C respectively.

According to the free proline content, the most stressed plants were those submitted to temperatures of 7°C (Table 2). The significant reduction in the levels of this indicator following spraying of a brassinosteroid is in keeping with the previously observed properties of this group of plant growth regulators (Sasse 1997, Núñez 1999, González-Olmedo et al. 2003). Similar results were obtained with the plants placed in chambers with the highest temperature and with the plants in the control group (27°C).

According to Sasse (1997), brassinosteroids play an independent role in the first stages of vegetative growth, in particular as growth promoters. Their characteristic is stimulating cellular elongation and division, vegetative growth, reproduction, interacting with other hormones, increasing yield and the production of biomass in different cultivars and accelerating maturation. Furthermore, they increase plant resistance to pests and different stress factors such as high salinity, drought, high temperature and powerful chemical agents such as pesticides and herbicides (Sasse 1997).

Cool temperatures affected leaf emergence, with a significant reduction in their number in comparison with the control (Table 3). The brassinosteroid analogue only resulted in an increase in this variable in plants placed in the chamber at 34°C.

None of the treatments resulted in significant changes in root numbers. Similarly, morphogenesis appears to be less affected than growth. Plant height was significantly reduced by both temperature extremes, whereas the application of brassinosteroids was effective only in plants exposed to the warmer temperature.

As regards fresh weight, temperature had no direct effect, with low values in all groups, except when sprayed with brassinosteroids in chambers at 27°C and 34°C, when there were significant increases. Brassinosteroids appear to exercise a major role on water regulation in treated plants, with no loss in the assimilation of photosynthates in control plants as demonstrated by the data in Table 3.

It is important to note the marked photosynthetic activity evident in the banana plantlets after only four weeks of acclimatization, as indicated by results in the chamber at 27°C. Extreme temperatures significantly reduced photosynthesis after 24 hours of exposure. The brassinosteroid had marked positive effects only at the highest temperature. In the week

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>BRAS (mg/L)</th>
<th>Survival (%)</th>
<th>% of plants with spots</th>
<th>Number of leaves with spots per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.1</td>
<td>97</td>
<td>100</td>
<td>97 b</td>
</tr>
<tr>
<td>7</td>
<td>0.0</td>
<td>97</td>
<td>100</td>
<td>97 a</td>
</tr>
<tr>
<td>27</td>
<td>0.1</td>
<td>100</td>
<td>100</td>
<td>0 e</td>
</tr>
<tr>
<td>34</td>
<td>0.0</td>
<td>97</td>
<td>100</td>
<td>38 c</td>
</tr>
<tr>
<td>34</td>
<td>0.1</td>
<td>100</td>
<td>100</td>
<td>18 d</td>
</tr>
</tbody>
</table>

Values followed by different letters in the same column are significantly different at p<0.05 according to Tukey’s test.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>BRAS (mg/L)</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.0</td>
<td>0.64 a</td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>0.23 bc</td>
</tr>
<tr>
<td>27</td>
<td>0.0</td>
<td>0.22 bcd</td>
</tr>
<tr>
<td>27</td>
<td>0.1</td>
<td>0.11 e</td>
</tr>
<tr>
<td>34</td>
<td>0.0</td>
<td>0.30 b</td>
</tr>
<tr>
<td>34</td>
<td>0.1</td>
<td>0.15 cd</td>
</tr>
</tbody>
</table>

Values followed by different letters in the same column are significantly different at p<0.05 according to Tukey’s test.
in which the acclimatization conditions were re-established, higher values of net photosynthesis can be seen in all groups, with highest photosynthetic activity in plants always maintained at 27°C and sprayed with brassinosteroids.

Regulation of stomatal opening is a complicated process dependent on many factors, including light, ambient CO₂ concentration, temperature, relative humidity, calcium concentration in the cytosol, hormones and enzymes, markers of related metabolic pathways, which also exercise very important effects and recoveries (Hussain et al. 1999, Casati et al. 2000, Li et al. 2001). Nevertheless, the results demonstrate that the gaseous exchange through the stomata in the experimental plants was favourable to those maintained at 27°C and furthermore that plants sprayed with brassinosteroids attained higher fresh weight values and photosynthetic activity.

As result of thermal stress, in particular that caused by low temperatures, the growth of the plantlets slowed down, similar to the behaviour of the physiological disorder known as choking (Daniells 1993), except for differences in age and development. Results with the brassinosteroid analogue suggest a potential for its use, pending further laboratory and field experiments.

Acknowledgements
To the memory of Dr Rodolfo Maribona who promoted this work.

References


One of the main problems that affects the production of Musa sp. in the tropics is black leaf streak disease, caused by Mycosphaerella fijiensis Morelet, a fungus recognized as the most aggressive form of the Mycosphaerella leaf spot disease complex. The disease has been reported to cause a 30% reduction in crop yield when the disease is not controlled and it is estimated that the cost of using fungicides to control the disease in Central America, Colombia and Mexico has reached 350 million dollars in eight years (Stierle et al. 1991).

Fungal pathogens are known to produce phytotoxins that are harmful to the host plant in low concentrations. These metabolites can diffuse from the site of infection to adjacent tissues causing necrosis, chlorosis, withering, or a combination of these symptoms, in a susceptible host (Wheeler 1981). Phytotoxins are classified as host-specific, or primary determinants of the disease, and non-host specific, or secondary determinants of the disease (Scheffer and Briggs 1981). The potential applications of phytotoxins include their use as probes to study the molecular basis of disease resistance and susceptibility in plants, as tools for the in vitro selection of disease resistant plant lines, and as possible agents for weed control (Buatti and Ingram 1991).

Most of our knowledge on phytotoxins comes from those produced by fungi of the Alternaria and Cochliobolus genus, but studies carried out on the fungal metabolites produced by M. fijiensis resulted in the identification of a number of phytotoxins, including the non-host specific fijiensin (Stierle et al. 1992, Upadhyay et al. 1991) and the host-specific tetralones (Stierle et al. 1991). However, attempts to generate disease resistant banana plants, using both semipurified fractions and pure phytotoxins from M. fijiensis as selection tools, have not been successful (Hareliman 1997, Okole and Schulz 1997).

These results suggest that neither fijiensin nor tetralones are essential for the pathogenicity of M. fijiensis and that the fungus might produce other metabolites that could be primary determinants of the disease. It is well known that some microorganisms producing metabolites under in vitro conditions do not produce them in vivo, or vice versa (Shaw 1991). Culturing conditions, e.g. medium composition, aeration and light, under which a fungus is grown, are the most important factors affecting the production of phytotoxins (Stierle et al. 1992). This study is part of a project aimed at isolating and identifying host specific phytotoxins produced by M. fijiensis. In order to determine the best culturing conditions for a high phytotoxicity of the fungal filtrates and a high yield of organic crude extracts, we evaluated the growth of M. fijiensis in eleven liquid media, under two lighting and two aeration conditions.

Materials and methods

The strain of M. fijiensis (W6) was provided by Dr. Andrew James of the Centro de Investigación Científica de Yucatán in Mexico. It was originally isolated from infected Musa acuminata plants.

A portion of the mycelium culture was placed in slant tubes containing 10 ml of potato dextrose agar (PDA) culture medium and the tubes were incubated for 30 days at room temperature and under a 12:12 photoperiod. After growth was completed, tubes were kept at 4°C until utilized.

An aqueous suspension of spores/mycelium was prepared by adding 2 ml of distilled sterile water to a slant tube containing a parent culture of M. fijiensis and removing the mycelium by using a wire. One ml of the aqueous suspension was used to inoculate a PDA plate, which was incubated for 20 days at room temperature under natural light conditions. Five ml of sterile water was added to each plate and a spore suspension was prepared by gently scratching the surface with a paintbrush. One ml of the spore suspension was used to inoculate a Roux flask containing liquid culture media.
Five liquid media, prepared as reported in the literature, were initially selected for evaluating the culturing conditions of *M. fijiensis*:

- V-8 juice medium (Peña-Rodriguez et al. 1988)
- Potato-dextrose broth (Natural 1989)
- Nutritious synthetic medium (Natural 1989)
- Czapek-Dox medium (Natural 1989)
- Synthetic M1D medium (Pinkerton and Strobel 1976).

However, since it has been reported that the addition of an infusion of the host plant to the culture medium promotes sporulation and phytotoxin production (Durbin 1981), the growth of *M. fijiensis* was also evaluated in a banana infusion medium prepared by adding 200 g of chopped banana leaves to 800 ml of boiling water and boiling for 30 min. After cooling, the mixture was filtered through cheesecloth and the volume adjusted to one liter. The infusion was then sterilized. Each of the liquid media was combined with a banana infusion in a 2:1 ratio. The cultures were evaluated under still and shaked conditions, and under total darkness and light/dark conditions.

For the still cultures of each culture medium, 250 ml of liquid medium were added to four 250 ml Roux bottles. Three of the flasks were inoculated with 1 ml of a *M. fijiensis* spore/mycelium suspension and the remaining flask was kept as control. The four flasks were maintained in the dark for 45 days, at 26±2°C. Similar preparations were also done using four Roux bottles that were kept under a 12:12 photoperiod for 45 days at 26°C.

For the shaked cultures of each culture medium, 300 ml of liquid medium were added to four 500 ml Erlenmeyer flasks. Three of them were inoculated with 1 ml of a *M. fijiensis* spore/mycelium suspension and the remaining flask was kept as control. Cultures were maintained in the dark at 100 rpm for 30 days, at 26°C. Similar preparations were also done using four Erlenmeyer flasks that were maintained under a 12:12 photoperiod for 30 days at 26°C.

Once the culture period was over, the culture broth was separated from the mycelial mat by filtration through two layers of cheesecloth. A 350 ml portion of the culture filtrate was lyophilized and resuspended in distilled water to a concentration of 100 mg/ml. The remaining filtrate was kept in a freezer.

**Leaf spot assay**

Leaves of susceptible banana plants (‘Grande naine’) were used to evaluate the phytotoxic activity. The leaves were obtained from plants growing in pots under greenhouse conditions.

The first or second youngest leaf of four-month-old banana plants was excised and disinfected for 60 seconds with a 5% sodium hypochlorite solution. The leaves were rinsed with distilled water, dried between two paper towels and placed in a plastic container, previously disinfected with 70% ethanol, lined with two layers of moist filter paper. The activity of the culture filtrates was tested by placing 20 µl of either the culture filtrate (3% and 1.5%), the uninoculated medium (3% and 1.5 %) or sterile water, on two incisions made with a scalpel on either side of the adaxial face of the leaf. Two leaves were utilized for each treatment and the containers were maintained at room temperature under natural light conditions. The effect (the area of the necrotic lesion) was registered at 24, 48 and 72 hours.

**Results and discussion**

Although both the still and shaked V8 cultures showed abundant mycelial growth under the two lighting conditions (data not shown), their yield of organic crude extract was higher under still and dark conditions (Table 1). Conversely, while the M1D cultures did not show discernible mycelial growth

<table>
<thead>
<tr>
<th>Culture medium*</th>
<th>12:12 photoperiod Still</th>
<th>12:12 photoperiod Shaked</th>
</tr>
</thead>
<tbody>
<tr>
<td>V8</td>
<td>21.3</td>
<td>83.0</td>
</tr>
<tr>
<td>Potato-dextrose broth</td>
<td>18.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Synthetic M1D</td>
<td>122.6</td>
<td>46.3</td>
</tr>
<tr>
<td>Nutritious synthetic</td>
<td><strong>--</strong></td>
<td><strong>--</strong></td>
</tr>
<tr>
<td>Czapek-Dox</td>
<td>11.0</td>
<td>11.6</td>
</tr>
</tbody>
</table>

* Only the media for which fungal growth was observed are listed.
** No significant growth observed.

Table 1. Quantity (mg/L) of organic crude extract obtained by culturing *Mycosphaerella fijiensis* under various conditions.
(data not shown), their yield of organic crude extract was consistently high (Table 1), in agreement with the high yields reported in the literature (Upadhyay et al. 1991). The results suggest that there is no relation between the amount of mycelium generated in a given culture medium and the yield of organic crude extract obtained.

It is interesting to note that neither the banana infusion nor any of the media containing banana infusion showed fungal growth. This suggests the presence of phytoalexins with antifungal activity in the culture medium, particularly in the banana infusion (Grayer and Kokubun 2001). The isolation and identification of the metabolites responsible for the antifungal activity is being done.

The strongest phytotoxic activities were observed in the filtrates of the V8 shaked cultures and the Czapek-Dox still cultures kept under light/dark conditions (Table 2). Given that the V8 shaked cultures kept under light/dark conditions had a higher yield of organic crude extract, and that many of the phytotoxic metabolites reported to date are readily extracted into organic solvents, these conditions were chosen as optimum for culturing *M. fijiensis*. Accordingly, we are presently working towards the isolation and identification of the phytotoxic metabolites produced by the fungus; the results of which will be published in due course.

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Table 2. Necrotic area (cm²) as a measure of the phytotoxic activity caused by 3% and 1.5% culture filtrates of *Mycosphaerella fijiensis* grown under different conditions and in various culture media.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Light conditions</th>
<th>Culture filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3%</td>
</tr>
<tr>
<td>Shaked cultures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V8</td>
<td>12:12 photoperiod</td>
<td>0.138</td>
</tr>
<tr>
<td>V8</td>
<td>darkness</td>
<td>0.207</td>
</tr>
<tr>
<td>Czapek-Dox</td>
<td>12:12 photoperiod</td>
<td>0.898</td>
</tr>
<tr>
<td>Czapek-Dox</td>
<td>darkness</td>
<td>0.243</td>
</tr>
<tr>
<td>Still cultures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V8</td>
<td>12:12 photoperiod</td>
<td>1.182</td>
</tr>
<tr>
<td>V8</td>
<td>darkness</td>
<td>0.237</td>
</tr>
<tr>
<td>Czapek-Dox</td>
<td>2:12 photoperiod</td>
<td>0.146</td>
</tr>
<tr>
<td>Czapek-Dox</td>
<td>darkness</td>
<td>0.267</td>
</tr>
</tbody>
</table>

* No activity.

References


Black leaf streak disease, caused by *Mycosphaerella fijiensis*, is the most destructive leaf spot disease of plantains and bananas. Yield losses between 20 to 90% have been reported (Stover 1983, Fouré 1985, Pasberg-Gauhl 1989, Mobambo et al. 1993).

Disease severity varies between cultivars, in part because of differences in virulence between the pathotypes (Fullerton and Olsen 1991). Consequently, the need to test on a given cultivar the aggressiveness of different *M. fijiensis* isolates has often been proposed. This, however, has been difficult to achieve because of the slow-growing nature of the fungus which precludes the production of enough inoculum for artificial inoculation (Mobambo 1993, Pasberg-Gauhl 1994). Moreover, most *M. fijiensis* isolates produce very few or no conidia at all in culture.

Allowing some air circulation is one of the most important factors for successful sporulation of various fungi (Henry and Andersen 1948, Lilly and Barnett 1950, 1951), a factor not investigated with *M. fijiensis* (Mobambo 1993, Pasberg-Gauhl 1994). This study was carried out to study the effect of light and of the sealing pattern on the sporulation and linear growth of *M. fijiensis*.

**Materials and methods**

Unexposed parts of stage 2 leaves (Ganry and Laville 1983, Gauhl et al. 1993) from an ‘Agbagba’ False horn plantain were cut into pieces of about 2 cm², placed on a solid potato dextrose agar (PDA) medium and autoclaved to form a PDA autoclaved plantain leaf (PAL) medium.

Three-month-old ascospore cultures derived from 30 *M. fijiensis* isolates were multiplied and subcultured on fresh PDA medium and incubated in a culture room at 25 to 29°C for 10 to 20 days. Three pieces of about 0.5 cm to 1.0 cm by 0.5 cm to 1.0 cm were cut from the actively growing mycelium of each isolate, placed in separate Petri dishes containing PAL medium and incubated for 21 days under continuous whitelight in a Gallenkamp incubator (INF-781-T) equipped with 8 fluorescent tubes (OSRAM, L8W/20). One of the three Petri dishes was sealed with parafilm throughout the incubation period, one was left unsealed throughout the incubation period, and one was sealed with parafilm for the first 14 days and unsealed for the next 7 days of incubation. The same procedure was repeated with the same isolates incubated for 21 days under blacklight, 2 Sylvania fluorescent lamp tubes (F40/350 BL) positioned 40.5 cm upward away from the culture dishes. Temperature was maintained between 26 to 29°C for each type of light.

The experiment was replicated four times. Linear colony size (the mean of the largest and smallest diameters out of a sample of 10) was measured at the end of the 21-day incubation period. Each colony was viewed under a Leitz larbolux S light microscope (20x) fitted with a Leica Wild MPS 52 camera to determine the number of sporulating isolates. Sporulation was expressed as a percentage that was arcsine-transformed (Gomez and Gomez 1984) and analysed by using Statgraphics version 2.1.

**Results and discussion**

Sporulation of *M. fijiensis* was observed to be dependent on both light and sealing pattern. Sporulation was significantly higher under blacklight than whitelight (Table 1). Exposure to UV light has been reported to induce sporulation in fungi (Ramsey and Bailey 1930, McCallan and Chan 1944), but recent studies have also shown that wavelengths between 300 to 380 nm, near the UV region, induce more sporulation than those in the UV range (200-300 nm). This latter region could be lethal or mutagenic (Leach 1971). Consequently, blacklight is preferred to whitelight in inducing sporulation of *M. fijiensis* isolates. Colony size was also significantly greater under blacklight than whitelight.

Sealing patterns were also observed to significantly (*P*<0.01) affect sporulation of *M. fijiensis* (Table 1). Sporulation was better in the Petri dishes that had been unsealed for the duration of the experiment or for the last 7 days. The accumulation of carbon dioxide...
and the production of inhibitory metabolic products such as ammonia could explain the lower level of sporulation in the airtight Petri dishes (Henry and Andersen 1948, Barnett and Lily 1950, Cochraine 1958). Although air circulation was an important factor, the percentage of sporulation was higher in the Petri dishes that had been sealed for the first 14 days of incubation and unsealed for the remaining 7 days than in those that had not been sealed for 21 days. This suggests that the metabolic products thought to accumulate in the sealed Petri dishes favour sporulation, but that their continuous presence has an inhibitory effect. Mean colony size, however, was significantly higher in Petri dishes left unsealed for the duration of the experiment.

Table 2 shows that blacklight significantly affected the growth of *M. fijiensis* colonies but only when the Petri dishes were sealed for 21 or 14 days. In the Petri dishes that were not sealed, linear colony size was not significantly different between the two types of light. The effect of aeration in enhancing the growth of *M. fijiensis* was more pronounced under whitelight than blacklight.

Further studies are needed to evaluate the effect of light and of the sealing pattern on the in vitro production of conidia. This may facilitate the testing of the aggressiveness of different pathotypes on different cultivars.

### References


### Table 1. Effect of light (under all sealing patterns) and sealing pattern (under both types of light) on the percentage of sporulation (original data and arcsine-transformed data that have been transformed back) and the mean linear colony size (diameter) of *Mycosphaerella fijiensis*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>Mean sporulation (%)</th>
<th>Transformed back arcsine data (%)</th>
<th>Mean linear colony size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White light</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blacklight</td>
<td>12</td>
<td>14.4</td>
<td>21.9 a</td>
<td>17.0 a</td>
</tr>
<tr>
<td>Sealed for 21 days</td>
<td>8</td>
<td>10.9</td>
<td>19.1 a</td>
<td>15.0 a</td>
</tr>
<tr>
<td>Unsealed for 21 days</td>
<td>8</td>
<td>19.9</td>
<td>25.9 b</td>
<td>21.4 c</td>
</tr>
<tr>
<td>Sealed for 14 days and unsealed for 7</td>
<td>8</td>
<td>29.5</td>
<td>32.3 c</td>
<td>17.0 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column are not significantly different at P<0.01 according to Duncan's Multiple Range Test.

### Table 2. Effect of light and sealing pattern on the percentage of sporulation (original data and arcsine-transformed data that have been transformed back) and the mean linear colony size (diameter) of *Mycosphaerella fijiensis* (n=4).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean sporulation (%)</th>
<th>Transformed back arcsine data (%)</th>
<th>Mean linear colony size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White light</td>
<td>8.9</td>
<td>17.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Sealed for 21 days</td>
<td>13.1</td>
<td>21.2</td>
<td>21.3</td>
</tr>
<tr>
<td>Sealed for 14 days and unsealed for 7</td>
<td>21.3</td>
<td>27.4</td>
<td>16.3</td>
</tr>
<tr>
<td>Blacklight</td>
<td>12.9</td>
<td>21.0</td>
<td>16.6</td>
</tr>
<tr>
<td>Unsealed for 21 days</td>
<td>26.7</td>
<td>31.0</td>
<td>21.5</td>
</tr>
<tr>
<td>Sealed for 14 days and unsealed for 7</td>
<td>37.6</td>
<td>37.3</td>
<td>17.6</td>
</tr>
</tbody>
</table>

Least significant difference at P<0.05

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Farm households grow *Musa* under widely varying conditions: under high to moderate rainfall or in irrigated desert environments, in poor to very fertile soils, and anywhere between sea level to altitudes of 1500 meters. Bananas are also grown under different cropping systems such as a monocrop, in association with annual or perennial crops, or in rotation with other crops. In each zone farmers face specific problems, including how to manage pests and diseases, and plant nutrition, which are all influenced by weather variability. In recent decades, new pests have been introduced and certain existing pests have been favoured by changes in cropping practices. Over the same time period, prices for agricultural products have fluctuated wildly, but new markets have also opened. As a result of all these factors, farmers make crop and pest management decisions under extreme uncertainty.

**What experts have offered farmers**

During much of human history, farmers have done their own research. Rural communities domesticated the major crops and designed a host of cropping systems. Only since the mid-1800s have governments, universities and scientists become involved in agricultural research, especially with the advent of fertilizers and pesticides (Staver 2001, 2003).

The approaches which scientists and development projects have offered farmers have evolved. During the early Green Revolution, plant breeders developed varieties responsive to fertilizers which they tested under various input levels to identify the best average performance. Farmers were expected to apply the same practices, including pesticides, year in and year out. In later years, scientists targeted their recommendations according to the soil and weather conditions and farmer resources.

The continuing search for greater productivity and lower environmental impacts has led to other approaches to improve production systems based on local variability.

**Precision agriculture.** Computerized spatial information on soils, drainage, yields and other characteristics is being used to fine tune the application of inputs for reduced environmental pollution and a more efficient yield response (Stoorvogel et al. 2004). However, predicting the yield response is made difficult by weather unpredictability while the costs of mapping and computer technology are justified primarily on large farms with high yield potential.

**Using local resources.** In response to the cost of purchased inputs, rural communities have begun to recover traditional technology and search for substitutes based on local resources, such as green manures and botanical brews, and soil conservation practices. Viewed at first with scepticism, these techniques are now being validated in scientific trials and incorporated into training programmes.

**Harnessing ecological processes in locally-designed cropping systems.** In this approach, farmers learn about ecological processes such as food webs, nutrient cycles, and habitat management and use these concepts to redesign and fine tune local cropping systems for pest suppression, more efficient nutrient cycling, and a healthier crop. The farmers are not simply following best average practice, but combine diverse strategies of crop and soil agro-ecosystem management (Figure 1). Based on their increasing knowledge of ecological processes and their ability to reason ecologically, they can innovate to manage variability and uncertainty and to use inputs that enhance ecological processes rather than substitute for them.

The increased recognition of the role played by variability has begun to broaden the focus of development projects from inputs and technologies to include the skills of the persons using the technologies, their information base for decision-making and their strategies for incorporating alternative practices. In a technological focus, scientific...
trials are used to generate and validate the practices which on average are the best. These are then promoted through field days (Figure 2A). However, from the perspective of the technology users, such field days are just one of many inputs into their decision making processes (Figure 2B). Farmers are experimenters and networkers. They filter information and experience from a wide range of sources and adapt them to their farming problems and opportunities. This switch from technology promotion to the strengthening of the farm community’s ability to adopt new technologies and integrate them into their livelihood strategy calls for alternative training approaches.

**Participatory group learning and experimentation**

Adult training differs from the formal education most of us experienced as children. Firstly, farmers participating in training are immersed in their subject. They have abundant experience producing the crop on their own farms with the resources available in their region under variable weather and fluctuating markets. Each farmer’s experience is a case of variability and therefore a resource for the trainer. Secondly, farmers are highly motivated to learn about a crop which is often part of their income and food security strategy, although they each have their own set of concerns. An adult training process should use daily experiences, experiments, information search, and network building. The interaction between the farmer and the trainer is two way and is facilitated by a process of learning and teaching through participatory group learning and experimentation.
life situations as learning laboratories, create a collaborative environment that emphasises the different experiences and approaches the participants bring and incorporate them in a learning cycle (analysis, planning, action, observation).

These principles were applied to an integrated pest management project in Nicaragua financed by Norway and run by the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) to come up with an approach for training farmers in agro-ecological crop and pest management known as participatory farmer group learning and experimentation by crop stage (Staver 2004). In the case of Musa, the project worked with MusaNic, a working group of research, teaching and extension organizations and grower associations dedicated to more effective research and training (CATIE 2003a). This approach draws on methods from FAO farmer field schools (www.communityipm.org) and farmer participatory research (Okali et al. 1994; Haverkort et al. 1991).

The objectives for the participants are to:
- learn about the ecology of the main pests and how to identify them;
- strengthen their observation skills linked to pest and crop management decisions;
- strengthen their agro-ecological reasoning capacity (food webs, life cycles, nutrient cycles, the role of abiotic and biotic factors in population dynamics, and the factors influencing the variability observed between farms, seasons and fields);
- increase their awareness of non-pesticide management alternatives;
- use experiments to test and adapt management alternatives;
- promote informal farmer networks;
- seek and evaluate information from many sources.

In participatory farmer group learning and experimentation by crop stage, an extensionist meets with a group of farmers before crop planting to discuss pest and crop problems and their experience with different practices, to prioritize problems and possible alternatives for testing, and to identify indicators for evaluating the group’s progress. Volunteers offer to establish experiments and to scout for pests in their fields. At key moments in the crop cycle, they meet to compare plant growth and pest levels. They also discuss the progress of their experiments. At each meeting they also conduct field visits and discovery exercises to strengthen their ecological knowledge of the crop, other primary producers, pests and beneficial organisms and soil factors. At the end of the cycle, the group reviews what they have discussed, seen, done and learned. They identify major lessons learned to be used in the next crop cycle and problems for further experimentation or study.

The approach uses participatory methods rather than conventional lectures with audiovisual materials for practical and philosophical reasons. In rural areas, electricity is often unreliable and equipment is expensive and difficult to transport. On the other hand, Musa fields of different ages with different pest problems are readily available for field laboratory exercises. Farmers are more comfortable in informal settings than in a classroom. In addition, retention and learning are more effective when active rather than passive methods are used.

The group approach multiples the learning opportunities for each participant. A group of 15-20 farmers meeting 4-6 times during a crop cycle may discuss up to 60-80 crop and pest scouting reports and 30-40 experiments.

The approach emphasises both learning and experimentation. Experimentation is a more formal process, while learning is much broader and can be achieved with many different approaches – comparisons of in-field variability, group analysis of monitoring data, farm visits, review of decision making at the end of the crop cycle, extrapolation of lessons learned from the small scale to the whole field and from putting into practice principles.

Finally, a training process by crop stage builds on numerous learning cycles that progress from observation and analysis to planning and action. The crop cycle itself is a learning cycle from which farmers learn for the next crop cycle. During the crop cycle, each farmer meeting is also a learning cycle based on the issues of the particular crop phase.

**Applying the approach to Musa**

The Pacific plains of Nicaragua have six months of rain and six months with minimal or no rain. This is not the ideal climate for Musa production, but plantains and cooking bananas are an important cash crop in
certain zones. The participatory farmer group learning and experimentation by crop stage in this region has seven meetings (Table 1) covering about 18 months (CATIE 2003a). Three events are planned prior to planting, one as a final evaluation and three during the crop cycle. Each event has a main theme corresponding to the decision making needs of the moment.

In the first meeting, farmers review their current production practices, the major problems they face and the alternatives they are testing. They also answer ten questions on their agro-ecological knowledge using live samples and photographs. As the meeting closes, they plan what they want to test in their fields and learn about the Musa agro-ecosystem.

The second meeting focuses on planting material, frequently a source of disease and pest infestations. Farmers walk a nearby field and gather plant samples that show problems. The group gathers to discuss what is the cause of each problem, how it reached the field, what causes it to increase and how it affects production. In the same field, farmers estimate the availability of poor, average and good planting material. They end the event by planting a small experiment with planting materials that have been pared or not. Volunteers offer to do the experiment on their own farm or to estimate sucker quality.

In the third meeting, farmers first report on the quality and number of suckers they found in the field from which they plan to obtain their planting material. In a nearby field that is at least in its third production cycle, small groups quantify disease and pest problems, plant vigour and bunch quality. Based on these data, they discuss possible measures to apply during planting to avoid the major problems found in old Musa fields. Next, they join the host farmer whose field is scheduled to be planted in the upcoming season. They map the field and its surroundings, identifying the factors conducive to certain problems, and then

Table 1. Example of a farmer training process in a wet-dry zone of Nicaragua.

<table>
<thead>
<tr>
<th>Crop stage</th>
<th>Theme and timing of meetings</th>
<th>Topics to be discussed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation for planting</td>
<td>Problems and priorities</td>
<td>1. How do we produce Musa?</td>
</tr>
<tr>
<td></td>
<td>(March - dry season)</td>
<td>2. What are our main problems?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. What do we know about Musa and its pests?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. What will we test/what do we want to learn?</td>
</tr>
<tr>
<td></td>
<td>Source of planting material</td>
<td>1. Diagnosing pests and diseases in new and old fields – origin of pests, their</td>
</tr>
<tr>
<td></td>
<td>(April - dry season)</td>
<td>movement, the conditions favouring their build-up</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Estimating how many suckers are available in each field</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Estimating sucker quality and preparation of planting material</td>
</tr>
<tr>
<td>Painting</td>
<td>Design of new field</td>
<td>1. Number and quality of suckers present in fields</td>
</tr>
<tr>
<td></td>
<td>(Early May – before the first rains)</td>
<td>2. Analysis of old fields (problems related to planting practices)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Analysis of the favourable and unfavourable factors in the new fields</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Evaluating the suckers planted at the previous meeting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. Possible experiments, including new cultivars</td>
</tr>
<tr>
<td>Vegetative growth</td>
<td>Status of new plants</td>
<td>1. Which experiments were set up</td>
</tr>
<tr>
<td></td>
<td>(August – rains)</td>
<td>2. Analysis of new plantings – effect of sucker and field characteristics on crop</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vigor and future pest problems</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Ideas for experiments – weeds, plant health, pest propagation</td>
</tr>
<tr>
<td>Harvest</td>
<td>Plan for next rains</td>
<td>1. Analysis of what is happening in experiments</td>
</tr>
<tr>
<td></td>
<td>(February - dry season)</td>
<td>2. Dry season soil cover and nutrient input</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Ideas and plans for rainy season management</td>
</tr>
<tr>
<td></td>
<td>Managing production</td>
<td>1. Analysis of what is happening in fields and experiments</td>
</tr>
<tr>
<td></td>
<td>(July – rains)</td>
<td>2. Evaluating plant and root health</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Projected harvest and costs</td>
</tr>
<tr>
<td></td>
<td>Final evaluation</td>
<td>1. Analysis of plant growth, yield and profits</td>
</tr>
<tr>
<td></td>
<td>(October – rains)</td>
<td>2. Analysis of whether the experiments addressed the identified problems</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. What was learned</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. What can be scaled up and what should be tested during next cycle</td>
</tr>
</tbody>
</table>
discuss management alternatives. They close the day by observing and then digging up the suckers planted in the previous meeting. They identify desirable suckers and discuss the effect of paring on sucker root growth and vigour. The extensionist and the farmers suggest alternative practices which might be tested.

In meetings four, five and six, the group monitors the progress of the plants, the increase in pest problems and the response of the crop to varying rainfall. They complete short learning exercises and set up experiments with alternative management. At each meeting, farmers also discuss the conditions in their own fields and report on the progress of their experiments.

At the evaluation meeting, the extensionist and the farmers review weather, pest levels and crop growth and yield during the cycle. They calculate costs and yields and discuss how to improve their decision-making. They repeat the test on their agro-ecological knowledge and reflect on what they have learned. They also analyse their experiments to determine if alternatives can be scaled up and what themes need attention in the next crop cycle.

**Designing a training for other zones**

Because farmers face a different array of problems depending on where they live, the content of the training process used in Nicaragua may not apply. In this section, we describe six broad steps which a group of trainers and scientists with practical experience in Musa can use to develop the curriculum for farmer participatory group learning and experimentation for their own conditions (CATIE 2003b).

1. **Define the main production zones by type of technology and agro-climate**

For the area of interest, the group identifies the range of production technologies used and the salient weather, climate and soil conditions under which the crop is grown. There may be two or three major production zones with a similar range of technologies. MusaNic in Nicaragua defined three zones (mid-altitude with a 3-month dry season, Atlantic lowlands with short dry season and Pacific lowlands with a 6-month dry season) and three production technologies (home consumption with minimal inputs and management, market orientation with limited inputs and no irrigation, market production with moderate inputs and irrigation).

2. **Identify problems in key zones**

Based on the experience of the assembled group of scientists and extensionists, each key zone is characterized according to the average yield, agronomic practices, main pest problems, extent of pesticide use, yield reducing problems, main production costs and particular problems which farmers face to achieve a greater value for their crop.

3. **Identify key alternatives to move from current to improved situation**

The group then describes an improved situation which farmers should be able to achieve without major changes in invested resources – perhaps yield increase of 30%, reduction in production cost of 25%, increased value of the crop of 40%, elimination of a toxic pesticide. The group identifies what needs to be done to achieve the improved situation, such as applying different cultural practices, acquiring new skills and doing additional research.

4. **Organize available information by crop stage**

With the information from the previous steps, the design group then lays out a matrix by crop stage. The group begins by identifying basic information by crop stage such as water and nutrient needs and the crop’s response to deficit and excess. Pest-crop interactions for each key pest can also be laid out by crop stage. How does the pest reach the field, what conditions favour and hinder its increase? Are there natural control organisms, what conditions favour and hinder their activity? Any relevant management practices leading to the elimination of toxic pesticides, a reduction in production costs or an increase in productivity are also identified. With this array of background information, the group then identifies the key moments when the farmers can meet for maximum learning. Many of the key decisions occur before planting.

5. **Identify what farmers will test out in their field**

At this stage in the design of the training curriculum, the group should identify which learning activities farmers might do when they return to their farms after each meeting. Trainers should bear in mind that
farmers will report on this activity at the next meeting. The activity might include monitoring pest levels, plant vigour or sucker quality, a learning exercise on sucker paring, debudding, estimating earthworm populations, weevil trapping, experimenting with alternative management practices such as new variety, green manure, planting density or selective weed management. The set of activities for the entire crop cycle should be closely linked to the key changes previously identified for moving the crop production from the current to an improved situation in terms of farmer skills and practices.

6. Design learning activities to motivate and prepare farmers to act
Once the group has identified the activities farmers could do, the meeting is designed to prepare farmers to return to their farms with the necessary skills, materials and enthusiasm to do the activity. A meeting of 4-5 hours should schedule time at the beginning to review, discuss and analyse what farmers have done since the last meeting and leave time at the end to identify who will do what before the next meeting. The middle section of 2-3 hours can be dedicated to new learning activities. In a training process with 6 farmer meetings, only about 8-12 hours will be available for these activities. The exercises must be carefully chosen and well designed for maximum learning value. Training events that are too long and complicated may be well intentioned, but are less effective than a well designed process with a moderate level of content.

Monitoring outcomes and impacts
The procedure described above prepares trainers and extensionists to work with farmer groups in a particular region. The design is a first approximation that can be modified during the process itself. After the diagnostic farmer meeting, activities can be adjusted based on the results of the test on agro-ecological knowledge and the prioritized problems. After each subsequent meeting, activities can continue to be adjusted based on the pest problems, or any other problem, that appeared during the crop cycle. The trainers and extensionists can also visit farmers in their fields between each meeting to verify the experiments, check their understanding of the training process and learn more about the resources and constraints of particular households.

By the end of the participatory group learning and experimentation process, the trainers will have numerous inputs to monitor the effectiveness of their work – how regular was farmer attendance, how much farmers learned, how many farmers did experiments, structured observation or learning exercises in their own fields and which practices farmers expect to scale up in the next planting cycle. This experience can then be used to modify the design of the new training cycle to more effectively strengthen farmer decision making in agro-ecological crop management (Staver and Guharay 2004).

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Although bananas and plantains (Musa spp.) are reputedly the fourth most important food staples in the world, until fairly recently these crops were largely ignored by national agricultural research institutes in developing countries. In the early 1980s, the long-established banana breeding programme in the Caribbean was dormant. Only Brazil, India, Nigeria, the Philippines and the East Caribbean had research programmes on Musa, albeit inadequately staffed and funded ones. Elsewhere, research on Musa was mainly the work of a handful of scientists working on individual research projects. The one exception was Honduras, where a private company had just handed over its breeding and pathology research programme on export bananas to the national government.

In developed countries, the Centre de coopération internationale en recherche agronomique pour le développement (Cirad) had the most important research programme on Musa with antennas in Montpellier, the Guadeloupe and French-speaking Africa. Australia had a smaller, but nevertheless important research programme.

A major reason for this relative neglect was the widespread misconception in the developed world that the banana was an export crop whose marketing and production is mainly the responsibility of large multinational companies, even though in the 1980s the proportion of the global Musa production that was exported as dessert bananas was, at 7%, even lower than today's. As is still the case, most of the bananas and plantains produced in the tropics are consumed locally.

Around the same time, however, scientists were cumulating achievements with the other major crops, and interest was gathering to develop, fund and coordinate actions on Musa at the international level. The main concerns were the narrow genetic base of the crop and the rapidly spreading black leaf streak disease, caused by the fungus Mycosphaerella fijiensis, that was devastating banana fields.

In Latin America and the Caribbean, which were free of black leaf streak disease at the time, the call for international assistance originated with ACORBAT, a regional association of scientists involved in research or extension work on Musa and their production systems. The Government of Jamaica also raised the need for international action at a meeting of the United Nations Conference on Trade and Development in November 1982. In West Africa, support came from the International Institute of Tropical Agriculture, which had established the West African Regional Cooperation for Research on Plantain (WARCORP) to conduct a series of collaborative projects in several countries with the aim of increasing the productivity of Musa in traditional farming systems. In Asia, the pressure for international action came from scientists at the University of the Philippines at Los Baños (UPLB). These Musa scientists had seen the impact on national programmes of the International Rice Research Institute (IRRI), also based at UPLB.

These regional interests directed their requests for a coordinated international approach and funding of Musa research to the International Development Research Centre (IDRC). In the previous decade, IDRC had shown considerable interest in initiating research on smallholder crops that had no home in the international agricultural research system.

Gathering support
IDRC responded positively and contracted a consultant to prepare a short briefing paper on the need for some form of action on Musa by the donor community. In November 1983, the paper was presented at the International Centres Week held in Washington. The meeting was attended by representatives from 15 donor agencies, 5 producing countries and 3 International Agricultural Research Centres. The participants agreed that the time had come for some form of international initiative to support the genetic improvement of

How INIBAP was born
A clear preference was expressed for a research network that would link donors with national programmes rather than an international agricultural centre similar to the ones that are members of the Consultative Group on International Agricultural Research (CGIAR). IDRC was asked to consult representatives of national Musa research programmes, Musa specialists, and donors, and to present a formal proposal at the meeting of the CGIAR donor group to be held in Rome in May 1984.

At the regional consultations for Africa, the participants expressed strong support for the network approach and a strong breeding component. After visiting, in early 1984, the Philippines, Thailand, Malaysia, Indonesia, Papua New Guinea and the South Pacific, where black leaf streak disease originated, scientists funded by the Australian Centre for International Agricultural Research reached the same conclusion about the need for an international Musa programme. At the regional consultations for Latin America and the Caribbean, which took place in Miami in April and May 1984, the participants made detailed suggestions in support of the network approach.

In addition to these regional consultations, three leading Musa experts were asked to advise on the broad shape of the research programme. Jean Champion, Norman Simmonds and Edmond de Langhe met at Gatwick, in the UK, in December 1983. They suggested that the main priorities were to identify and evaluate banana clones in producing countries; to gain a socio-agricultural understanding of Musa production systems and their utilization; and to develop an international breeding programme aimed primarily at the smallholder crop. Structurally the group foresaw the need for an international breeding programme, three regional networks and a sound governance ensured by a small secretariat supported by a scientific advisory body.

A number of meetings bringing together representatives of interested donor countries and agencies were also organized by the IDRC consultant. Most of these focused on structural matters. Of particular concern to some donors was the localization of the central secretariat. This had also been an issue during the regional consultations, at which a number of participants had suggested their country as a suitable place to set up the headquarters of the network.

**Working out the last details**

The briefing paper proposing the establishment of a network was presented to the donor group meeting in Rome in May 1984. The donor group approved the paper in principle and asked IDRC to undertake more consultations to obtain funding commitments from donors, determine the localization of the headquarters and the membership of the Board of Trustees and provide a list of potential Directors Generals in time for Centres Week in November 1984. At the meeting, the recommendation to create a network backed by a donor support group, rather than a CGIAR centre, was accepted, in light of the constraints on CGIAR funding and the number of institutions vying for CGIAR membership.

At the first meeting of the donor support group, an agreement was reached on the composition of the Board of Trustees, the appointment of Edmond de Langhe as Director and the name of the new institute, the International Network for the Improvement of Banana and Plantain (INIBAP), which was to be located in Montpellier. Some donors pledged funds for the first year of operations and IDRC agreed to be the executing agency until the institute was up and running.

The next step was the signing with the Government of France of an international treaty granting INIBAP an international status. Signed in December 1988, the treaty had to be ratified by at least four other countries. By 1990, it had been ratified by Belgium, Canada, Colombia, the Philippines and Senegal.

INIBAP operated as an autonomous and nonprofit intergovernmental organization until 1990, when it was invited to join the CGIAR system. The next turning point was 1995, when INIBAP joined the International Plant Genetic Resources Institute (IPGRI), another member of the CGIAR.
The results are in. After years of work, the ploidy levels of all the accessions in the world’s largest collection of *Musa* germplasm are known.

The International *Musa* Germplasm Collection is held at the INIBAP Transit Centre (ITC), which is hosted by the Katholieke Universiteit Leuven (KULeuven) in Belgium. The accessions are maintained under slow growth conditions in the form of proliferating shoot tips (Van den houwe et al. 1995). The ITC currently stores 1175 accessions, each of which is represented by 20 tissue culture plantlets. A list of the accessions available in the collection can be found on the INIBAP website (http://www.inibap.org).

As part of its mandate to facilitate access to genetic diversity, the ITC also distributes samples free of charge to bona fide users, together with the data available on the accessions. Over the past 20 years, it has provided more than 60 000 samples of virus-free accessions to researchers worldwide. In order to stimulate a still wider use of the accessions, in the spring of 1999, the ITC undertook the task of determining the ploidy level of all the accessions in the collection using the best available technique.

Ploidy, or the number of chromosome sets in a cell, is one of the defining characters of a species or cultivar. Wild species and subspecies are all diploid, whereas cultivars can be diploid, triploid or tetraploid. Knowing the ploidy level of an accession is not only useful to confirm its classification, but it can also reveal whether the plant has been changed by *in vitro* conservation (*in vitro* storage has been implicated in mutations, epigenetic changes and changes in chromosome structure and number).

Ploidy has conventionally been determined by counting chromosomes in dividing root tip cells, a labour intensive procedure that is made difficult in *Musa* by the fact that the chromosomes are small and numerous. A further disadvantage of this approach is that the number of chromosomes in the root cells is not necessarily representative of the one in the rest of the plant.

About ten years ago, the Laboratory of Molecular Cytogenetics and Cytometry at the Institute of Experimental Botany (IEB) demonstrated that the ploidy level of banana plants could be accurately determined by using flow cytometry (Dolezel et al. 1994). Flow cytometry measures the content of nuclear DNA, which is directly proportional to the number of chromosomes. More recently, this technique has also been successfully used on *Musa* embryogenic cell suspensions (Roux et al. 2004). Since preparing the sample is easy and the analysis is performed in just a few minutes, the method is suitable to screen a large number of plants. Unlike chromosome counting, cytometry can be performed with any type of tissue, a feature that facilitates the detection of mixoploidy, i.e. plants that contain cells of different ploidy levels.

**The tally**

At the end of the project in 2004, the 1150 accessions held in the collection had been analysed (25 accessions have since been added to the collection). Since the IEB is located some 1000 km east of Leuven, in Olomouc, Czech Republic, *in vitro* rooted plants were shipped in Cultusaks® (Figure 1) in batches of 50 accessions, with five plants per accession. Upon arrival, the plants were transplanted and maintained in a greenhouse for up to eight weeks until their analysis. A small piece (about 50 mg) of young leaf tissue was used to prepare the nuclei suspension to be analysed by flow cytometry.
cytometry (Dolezel et al. 1997). At least four plants per accession were analysed. The accessions for which the results were not consistent were re-analysed and the sample size increased to at least 10 plants.

The data are now available in the INIBAP Musa Germplasm Information System (MGIS), a database holding the characterization and evaluation data of the Musa accessions maintained in 16 banana collections around the world. The data are accessible online at http://mgis.grinfo.net under cytological characters.

The analysis confirmed the ploidy of 958 accessions and revealed the level of 81 accessions for which it was unknown (Figure 2). Not only did this work support the ploidy classification, it also confirmed that maintaining plants under in vitro conditions does not lead to large-scale changes in the genome. In nearly 10% of the accessions, however, ploidy turned out to be different from the previously accepted level. For example, the cultivar ‘Kamaramasenge’ (ITC0127) is now classified as a triploid rather than a diploid (Table 1).

Mixoploidy, a clear sign of genetic instability, was observed in nine accessions such as ‘Auko’ (ITC0987), which was found to consist entirely of mixoploid plants containing both diploid and triploid cells (Table 2). The other accessions in which mixoploidy was detected, were also represented by non-mixoploid plants whose ploidy level was different from the expected one.

Finally, 14 accessions had no mixoploid plants but were represented by plants with different ploidy levels, such as ‘Ibwi’ (ITC1465), which had 8 diploid plants and 8 triploid plants (Table 2). Such accessions, whose difference in ploidy level cannot be attributed to polyploidy (a doubling of the genome), represent 85% of the accessions that ended up with a ploidy level different from the expected one. One possible explanation is that ploidy was incorrectly determined the first time around. Another is an accidental interchange of plants from different accessions during the subculturing process.

To our knowledge, this work is the largest exercise ever undertaken to determine the ploidy levels of a collection of vegetatively propagated crop. The results also provide quantitative data on the extent of genetic variation in plant materials maintained under in vitro conditions. As to the nature and origin of the variation observed, these questions will be answered by the ongoing efforts to characterize the accessions by using molecular markers and by planting the accessions in the field to evaluate them in situ. It is also planned to assess the ploidy of each incoming accession.

Acknowledgements

We thank our colleagues Jan Bartoš, Nikol Gasmanová, Pavlína Kovárová, Martin Lysák, Pavla Suchánková, Katerina Vlácilová and Jan Vrána for the flow cytometric analyses, and Els Kempeaers, Ronald Marie Dolezelová and Jaroslav Dolezel work at the Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, CZ-77200 Olomouc, Czech Republic, Ines Van den Houwe at the INIBAP Transit Centre, Kasteelpark Arenberg 13 - 3001 Leuven, Belgium, Nicolas Roux at INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France, and Rony Swennen at the Laboratory of Tropical Crop Improvement, KULeuven, Kasteelpark Arenberg 13 - 3001 Leuven, Belgium.

Table 1. Examples of accessions in which the expected ploidy was not confirmed.

<table>
<thead>
<tr>
<th>ITC Code</th>
<th>Accession name</th>
<th>Group</th>
<th>Expected ploidy</th>
<th>Ploidy estimated by flow cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>0127</td>
<td>Kamaramasenge</td>
<td>AB</td>
<td>2x</td>
<td>2x</td>
</tr>
<tr>
<td>0051</td>
<td>Foulah 4</td>
<td>ABB</td>
<td>3x</td>
<td>4x</td>
</tr>
<tr>
<td>1261</td>
<td>PA03-22</td>
<td>AAAB</td>
<td>4x</td>
<td>3x</td>
</tr>
<tr>
<td>1065</td>
<td>Pisang slendang</td>
<td>AABB</td>
<td>4x</td>
<td>3x</td>
</tr>
<tr>
<td>1027</td>
<td>Asupina</td>
<td>Fe’i</td>
<td>2x</td>
<td>3x</td>
</tr>
</tbody>
</table>

Table 2. Examples of accessions in which mixoploid plants and plants that turned out to have a ploidy level different from the expected one were found.

<table>
<thead>
<tr>
<th>ITC Code</th>
<th>Accession name</th>
<th>Group</th>
<th>Expected ploidy</th>
<th>Ploidy estimated by flow cytometry*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0983</td>
<td>Auko</td>
<td>AB</td>
<td>2x</td>
<td>2x+3x (15)</td>
</tr>
<tr>
<td>0987</td>
<td>Auko</td>
<td>AB</td>
<td>2x</td>
<td>2x (10); 2x+3x (13)</td>
</tr>
<tr>
<td>0796</td>
<td>Kirkiman</td>
<td>AA</td>
<td>2x</td>
<td>2x (10); 4x (2); 2x+4x (2)</td>
</tr>
<tr>
<td>0043</td>
<td>Champra nasik</td>
<td>AAAAA</td>
<td>4x</td>
<td>2x (7); 4x (10)</td>
</tr>
<tr>
<td>1465</td>
<td>Ibwi</td>
<td>AAh</td>
<td>3x</td>
<td>2x (8); 3x (8)</td>
</tr>
</tbody>
</table>

*The number of plants is given in parentheses.

Figure 2: Distribution of 1150 Musa accessions in relation to their ploidy level before and after flow cytometry analysis. Mixoploidy refers to a plant containing cells of different ploidy (e.g. 2x+3x). Mixed ploidy refers to accessions represented by plants of different ploidy.

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Bogaerts and Jeroen Mertens for the in vitro manipulations. This work was supported by INIBAP (research contracts INIB 98/21 and INIB 2001/06) and by the International Atomic Energy Agency (research contract No. 12230) and was conducted within the framework of PROMUSA, a Global Programme for Banana and Plantain Improvement.

References

Results of survey on IPGRI’s commodity work

In December 2004, a stakeholder survey was conducted as part of IPGRI’s commissioned external review of its new Commodities for Livelihoods Programme. The survey comprised 31 questions in three languages aimed at drawing comment on various aspects of IPGRI’s work on cacao, coconut, and banana and plantain: the coordination of networks, information products, quality of partnerships and future priorities. Approximately 300 participants were asked to complete the survey and an open invitation to take part in the survey was posted on the INIBAP web site. In total, 128 people responded by completing the survey either on the Internet or by sending it through e-mail.

Most of the respondents said being in regular contact with IPGRI (twice or more a year) and being employed in public-funded research institutes (62%). Three-quarters were biological or agricultural scientists. Each banana-producing region had roughly equal numbers of respondents. Members of networks, including regional networks, PROMUSA and the Global Musa Genomics Consortium, made up 64% of the respondents, of which around one-third belonged to more than one network. However, only 7 people responded from the two African regional networks.

The following brief summary provides those findings from the survey that relate to INIBAP:

Networks
When asked how adequately networks have served a function, respondents replied that they had served or partly served a large range of uses, most significantly providing a forum for meetings. Responses in some areas gave a clear indication of where network members consider improvements should be made (Table 1).

Members of all networks indicated that “finding funds from external sources” was a high priority to make networks function more effectively (Table 2). By contrast, there were mixed responses to the idea of restricting or expanding membership, and a large number of respondents (40%) were not in favour of “asking members to pay fees or in-kind contributions”.

When asked whether IPGRI was a good partner to work with, 80% agreed and 17% partly agreed. The large majority of partners expressed a positive impression of their working relationship with IPGRI (Table 3).

The majority of respondents indicated that IPGRI had performed adequately its

Table 1. Percentage of respondents who considered that the network served the various functions adequately (as opposed to needing improvement or development). Figures in bold indicate the largest proportion of respondents.

<table>
<thead>
<tr>
<th>Network functions</th>
<th>PROMUSA (31 respondents)</th>
<th>Musa Genomics (9 respondents)</th>
<th>Regional banana networks (34 respondents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Providing a forum for meetings</td>
<td>68%</td>
<td>67%</td>
<td>68%</td>
</tr>
<tr>
<td>Developing priorities at a regional/global levels</td>
<td>29%</td>
<td>44%</td>
<td>50%</td>
</tr>
<tr>
<td>Capacity building/training</td>
<td>27%</td>
<td>11%</td>
<td>28%</td>
</tr>
<tr>
<td>Establishing complementarities among organizations</td>
<td>10%</td>
<td>22%</td>
<td>42%</td>
</tr>
</tbody>
</table>
obligations in coordinating projects (e.g. administration, communication, planning, monitoring and evaluation, developing shared aims and visions, establishing decision-making that is fair etc.). Three areas were highlighted by a majority of respondents considering improvement or development to be necessary in INIBAP’s project coordination: “establishing effective mechanisms for managing data or results” (54%), “planning” (51%), and “establishing an effective framework for project monitoring and evaluation” (50%).

All INIBAP information products, including databases and web sites, were considered “very useful” or “quite useful” by 75% or more of the respondents. INFOMUSA attracted the strongest response with 75% of respondents considering it to be “very useful”.

**Future priorities**

The large majority of respondents (84-96%) considered that IPGRI’s existing and proposed activities in conservation were medium or high priority: “providing a network for germplasm conservation and management” and “building capacity to conserve and distribute germplasm in NARS and other partners” were the most popular responses (Table 4).

There was more divergence in opinion concerning genetic research and improvement activities. Although a majority of respondents considered research into genetic manipulation and embryogenic cell suspensions to be medium or high priority, a notable percentage considered it to be “not a priority” (26% and 21% respectively). Research in genomics and bioinformatics received slightly more support (Table 5).

As with genetic improvement, there is strong support for promoting the use of biodiversity in production systems (Table 6). There is more ambivalent support for less conventional areas of development work (e.g. strengthening community based organizations, developing processing technologies and promoting enterprise development).

**Conclusions**

The survey has provided valuable feedback to assist INIBAP in confirming where its strengths lie, in suggesting where improvements in networking are demanded and in attaining stakeholders’ perspectives.

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**Table 2. Percentage of respondents considering the various mechanisms to be high priority (as opposed to medium, low or not a priority). Only the mechanisms considered as high priority by more than 70% of respondents are presented. Figures in bold indicate the largest proportion of respondents.**

<table>
<thead>
<tr>
<th>Mechanisms for rendering networks more effective</th>
<th>PROMUSA (32 respondents)</th>
<th>Musa Genomics (9 respondents)</th>
<th>Regional banana networks (32 respondents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finding funds from external sources</td>
<td>88%</td>
<td>89%</td>
<td>90%</td>
</tr>
<tr>
<td>Collecting baseline information for the development priorities and decisions</td>
<td>81%</td>
<td>22%</td>
<td>68%</td>
</tr>
<tr>
<td>Providing incentives for information exchange</td>
<td>72%</td>
<td>78%</td>
<td>58%</td>
</tr>
<tr>
<td>Developing data management tools</td>
<td>61%</td>
<td>33%</td>
<td>71%</td>
</tr>
<tr>
<td>Involving members in functioning and coordination of the network</td>
<td>52%</td>
<td>22%</td>
<td>72%</td>
</tr>
</tbody>
</table>

**Table 3. Percentage of respondents who strongly or partly agreed or disagreed with various statements concerning partnerships with IPGRI.**

<table>
<thead>
<tr>
<th>Characteristics of the partnership with IPGRI</th>
<th>Strongly agree</th>
<th>Partly agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPGRI is a good partner</td>
<td>80%</td>
<td>17%</td>
</tr>
<tr>
<td>IPGRI develops trust and shared interests with its partners</td>
<td>66%</td>
<td>31%</td>
</tr>
<tr>
<td>IPGRI is a communicative partner</td>
<td>59%</td>
<td>35%</td>
</tr>
<tr>
<td>IPGRI works under equal partnership with my organization</td>
<td>49%</td>
<td>36%</td>
</tr>
</tbody>
</table>

**Table 4. Percentage of respondents considering the various germplasm conservation, exchange and evaluation activities to be high priority (as opposed to medium, low or not a priority). Figures in bold indicate the largest proportion of respondents.**

<table>
<thead>
<tr>
<th>Germplasm conservation, exchange and evaluation</th>
<th>Proportion of 72 respondents who considered activities to be high priority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Network for germplasm conservation and management</td>
<td>74%</td>
</tr>
<tr>
<td>Building capacity in NARS and partners to conserve and distribute germplasm</td>
<td>74%</td>
</tr>
<tr>
<td>Collecting wild or cultivated germplasm</td>
<td>67%</td>
</tr>
<tr>
<td>Ensuring long term security of ex situ collections</td>
<td>67%</td>
</tr>
<tr>
<td>Conserving germplasm in situ or on farm</td>
<td>64%</td>
</tr>
<tr>
<td>Research on diseases and therapy for ex situ collections</td>
<td>62%</td>
</tr>
<tr>
<td>Providing incentives for germplasm exchange</td>
<td>61%</td>
</tr>
<tr>
<td>Acting on genetic erosion</td>
<td>57%</td>
</tr>
<tr>
<td>Molecular characterization</td>
<td>54%</td>
</tr>
<tr>
<td>Research on ex situ conservation methodologies</td>
<td>52%</td>
</tr>
</tbody>
</table>

**Table 5. Percentage of respondents considering the various genetic research and improvement activities to be high priority (as opposed to medium, low or not a priority). Figures in bold indicate the largest proportion of respondents.**

<table>
<thead>
<tr>
<th>Genetic research and improvement activities</th>
<th>Proportion of 71 respondents who considered activities to be high priority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research on pest and disease resistance</td>
<td>86%</td>
</tr>
<tr>
<td>Use of biodiversity in genetic improvement</td>
<td>76%</td>
</tr>
<tr>
<td>Supporting breeding programmes</td>
<td>76%</td>
</tr>
<tr>
<td>Building capacity to carry out genetic research &amp; improvement in NARS</td>
<td>71%</td>
</tr>
<tr>
<td>Pooling research resources</td>
<td>68%</td>
</tr>
<tr>
<td>Strengthening networking</td>
<td>61%</td>
</tr>
<tr>
<td>Producing guidelines</td>
<td>55%</td>
</tr>
<tr>
<td>Research in genomics</td>
<td>53%</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>48%</td>
</tr>
<tr>
<td>Research in genetic manipulation</td>
<td>35%</td>
</tr>
<tr>
<td>Research in embryogenic cell suspensions</td>
<td>36%</td>
</tr>
</tbody>
</table>
of where our priorities lie. We would like to thank wholeheartedly those who took part in the exercise and assure them that their input has been registered and valued, by the review panel of the CCER and IPGRI’s Board of Trustees as well as by the staff at INIBAP. We hope that we can continue to depend on their support.

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### Highlights from the reader survey

**Who are the respondents?**
A total of 326 respondents, or 12% of our individual subscribers, returned the questionnaire. Of these, 54% filled in the English version, 35% the Spanish one and 11% the French one, a distribution that more or less reflects the proportion of INFO MUSA printed in each language. The greatest contingent of respondents is based in the Latin America and Caribbean region (41%) followed by Africa (27%), Asia (19%), Europe (7%), the Pacific Islands (3%), North America (1.5%) and the Middle East (1.5%). A little over 60% of the respondents are scientists. Most of the people who returned the questionnaire (76%) are subscribers. The others access it from the Internet (11%) or from a library (8%), borrow it from a colleague (2%) or get it through some another unspecified way (3%).

**How do you rate INFO MUSA?**
Most of the respondents think that INFO MUSA is a useful source of information and appreciate its format and contents: 80% of the respondents rate its usefulness in keeping up with research on Musa as strong and the remaining 20% as average, while the quality of the writing and of the presentation were respectively rated as strong by 72% and 76% of the respondents and as average by 28% and 22% of the respondents. The quality of the scientific content was rated as strong by 65% of the respondents and as average by 32%.

**What kind of INFO MUSA?**
Only a few respondents would not mind if INFO MUSA was published only in English and available only electronically: being available in print was rated as very important by 74% of the respondents, and important by 25%, and being available in three languages was rated as very important by 65% of the respondents, and important by 31%.

**Should INFO MUSA be a recognized peer-reviewed journal?**
A majority of respondents (69%) would like to see INFO MUSA become a peer-reviewed journal. When broken down by region, the results show that the greatest proportion of respondents in favour of such a move (47%) are based in Africa (82% of the respondents from this region said yes) followed by Latin America (68% of the respondents from this region said yes). Asians were split 50:50 on the question, whereas only 17% of the European respondents and none of the respondents from North America and the Pacific Islands think INFO MUSA should become a peer-reviewed journal.

**Which sections do you read and how often?**
Eighty-seven percent of the respondents always read the scientific articles, 80% Musanews, 65% the Focus on section, 60% the thesis abstracts and 47% the editorial. The vast majority of the remaining
respondents said they sometimes read these sections.

**Are you satisfied with the spectrum of articles?**

Only a minority of respondents would like to see more articles on genetic transformation and germplasm screening (31%), molecular biology (34%) and tissue culture (40%). The greatest demand was for articles on integrated pest management (62%), pests and diseases (60%) and uses and products (54%). Many of the respondents who answered the question about the type of information they would like to see added were also mainly interested in seeing more practical information and articles on applied research.

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**Variability in reproductive fitness and pathogenicity of *Radopholus similis* in *Musa*: effect of biotic and abiotic factors**

*Thomas Moens*

**PhD thesis submitted in October 2004 to the Katholieke Universiteit Leuven, Belgium**

Nematodes are one of the most important constraints in *Musa* production after leaf spot diseases. These tubular, veriform animals, especially *Radopholus similis*, parasitize the root system and can reduce yield by up to 80%. Nematodes induce root damage directly, and indirectly by facilitating the entry of fungi and bacteria. This results in lower nutrient uptake, extends the harvest-to-harvest interval and increases plant toppling, when the plant is not propped up. This research was conducted in Costa Rica at the Corporación Bananera Nacional (CORBANA).

The population growth of 11 *R. similis* populations extracted from roots of plants in a commercial plantation, and of 7 *R. similis* isolates extracted from the roots of one plant in a field of 4.5 ha, was evaluated on carrot disks and ‘Grande naine’ potted plants. Differences in reproductive fitness among the populations and isolates were observed on carrot disks and in potted plants. Variations were expected among the populations but not among the isolates. These may be due to the presence, among the populations or isolates, of subpopulations better adapted to growth on carrot disks, to the number of subcultures on carrot disk, or to differences in female reproductive fitness. Under our experimental conditions, no relation was found between reproductive fitness and pathogenicity, as reported in the scientific literature.

To screen the resistance of *Musa* varieties to *R. similis*, parameters like inoculum number, substrate type, nematode population, exposure time and pot volume are important. However, published literature show that these parameters are not standardized. Therefore, the effect of each of these factors on reproductive fitness and pathogenicity were studied to optimize screening protocols. Cavendish plants grown in river sand supported the highest *R. similis* numbers and had the lowest root weight, compared to different banana soils that varied in sand, silt or clay content. This was probably due to a higher macroporosity and a better aeration. When comparing exposure times between 2 to 16 weeks, the highest increase in *R. similis* population was observed between 6 and 12 weeks after inoculation. Root weight of Cavendish plants grown in pots of 4 different volumes, and inoculated with identical densities, was significantly higher in 1.8 and 3.6 L pots, compared to 0.4 and 0.8 L pots. Application of these factors in a time experiment confirmed the susceptibility of ‘Grande naine’ and resistance of ‘Yangambi km 5’ and FHIA-23 to *R. similis*. Based on these results, *Musa* plants grown in local soil, initially inoculated with 500 *R. similis* and exposed during 8 to 12 weeks in 1.8 L pots, gave consistent screening results.

Thereafter, the protocol was applied for resistance testing against *R. similis*. Twenty-six *Musa* varieties, belonging to different genome groups (AA, AB, BB, AAB, ABB, AAAA, AAAB and AABB) and 10 F1 lines
of a cross between *Musa acuminata* ssp. *burmannicoides* 'Calcutta 4' (AA) and 'Pisang Berlin' (AA) were tested for resistance to *R. similis* under *in vitro* conditions and in pot plants. *In vitro*, no difference in final nematode numbers was found among the tested varieties, including 'Grande naine', 'Pisang jari buaya' and 'Yangambi km 5', 30 days after inoculation. In contrast, 'Yangambi km 5', FHIA-23, 'Kunnan', 'Paka' and 'Pisang lemak manis' in pots supported lower nematode numbers, compared to 'Grand naine', 8 weeks after inoculation. The longer exposure time probably allowed the expression of the resistance mechanisms in these varieties grown in pots. Also 'Tjau lagada' proved, for the first time, to be resistant to *R. similis*. These results need to be confirmed under different soil and climatic conditions in the field.

*Helicotylenchus multicinctus*, *Meloidogyne incognita* and *Pratylenchus coffeae* were studied in pot and microplot conditions. In pots, *H. multicinctus* suppressed root weight, while *M. incognita* stimulated it and *P. coffeae* did not influence it over the tested time intervals. In a pot experiment, the mean number of *R. similis* in plants that had been inoculated with *M. incognita* were always lower than in noninoculated plants. This illustrates the suppressive impact of this nematode on *R. similis*, probably due to a reduction in feeding sites. *H. multicinctus* and *M. incognita* numbers were not affected by increased *R. similis* inoculum numbers, while this was the case for *P. coffeae*. The suppressive effect of *R. similis* on *P. coffeae* can be due to competition for the same feeding site, *i.e.* the root cortex.

In the screening experiments, 'Yangambi km 5', 'Niyarma yik' and 'Pisang Berlin' supported the lowest numbers of *H. multicinctus* per 100 g of roots, together with 'Pisang bungai' and 'Tjau lagada'. All tested varieties proved to be susceptible to *M. incognita*, while FHIA-01 and FHIA-18 were equally resistant to *P. coffeae*, compared to 'Yangambi km 5', a variety with known resistance to this nematode. Also 'Tjau lagada', 'Pisang mas' and 'Pisang bungai' were less susceptible to *P. coffeae* than 'Grande naine'. These characteristics of 'Tjau lagada', together with its resistance to *R. similis* and its partial resistance to black leaf streak disease, make this variety interesting for further research.

In a microplot experiment using 200 L drums filled with sterilized soil, inoculated 'Grande naine' plants were followed until harvest and compared with uninoculated plants. Only *R. similis* significantly reduced root weight by 66%, while root damage was higher and bunch weight was lower in *R. similis* and *P. coffeae* inoculated plants, compared with the control. Also *M. incognita* reduced bunch weight. Probably the detrimental effect of *M. incognita* is more associated with physiological alterations, changing the cell structure and metabolism, than physical root deterioration. Due to their negative effect on roots or yield, not only *R. similis*, but also *P. coffeae*, *M. incognita* and *H. multicinctus* have to be considered in future breeding programmes for nematode resistance.

Finally, the biodegradation of 6 nematicides was studied alone or in rotation, under field conditions and in a *R. similis*-maize laboratory test. In the field, only terbufos (Counter®) consistently reduced the number of nematodes per 100 g of roots after 5 consecutive applications. Carbofuran (Furadan®), ethoprophos (Mocap®), fenamiphos (Nemacur®) and the untreated plants supported the highest numbers of nematodes per 100 g of roots. The mean percentage of functional roots for all nematicide treatments was 7% higher than in the untreated plants. This is reflected in a 38% higher bunch weight of the treated plants. Of all nematicides, only carbofuran resulted in a significantly lower bunch weight, which was still higher than the one of the untreated plants. Nevertheless, carbofuran, ethoprophos, fenamiphos and oxamyl showed enhanced biodegradation in the biotest after 5 consecutive field applications. Contrasting results are probably due to the variation in the start of enhanced biodegradation for the tested nematicides and the slow root health deterioration. Nematicide rotation gave the highest bunch weight and a high proportion of functional root, because of the low probability of developing enhanced biodegradation. Further research is needed to study the development of enhanced biodegradation after increasing nematicide applications in different soil conditions. Nevertheless, due to the future ban on nematicide use in banana, the development of alternative nematode management techniques is urgent.
Breeding for resistance to *Radopholus similis* in East African highland bananas (*Musa* spp.)

Carine Dochez

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The East African highland bananas (*Musa* spp. AAA) are the most important staple food crop in the East African Great Lakes Region. East Africans cook the fruit as ‘matooke’ or brew beer from it. In Uganda, East African highland bananas are divided in five clone sets: four clone sets cover the cooking types (Nfuuka, Nakitembe, Nakabululu and Musakala) and one clone set covers the beer brewing types (Mbidde).

Uganda is the leading regional producer and consumer. Until the 1970s, East African highland bananas were traditionally grown in central Uganda. However, since that time, banana production has declined by more than 25%. This decline has led to the replacement of cooking bananas by exotic banana cultivars and annual food crops. At the same time, cultivation of the crop has shifted to the southwest of the country.

Nematodes are considered one of the major constraints causing this decline. The burrowing nematode, *Radopholus similis*, has been identified as the most destructive species in Uganda.

Nematodes can be reliably controlled by nematicides. However, the use of nematicides has adverse environmental effects and is too costly for resource-poor farmers. A promising alternative is the use of nematode resistant cultivars. Improving the East African highland bananas through breeding was identified by the Ugandan National Agricultural Research Organisation (NARO) as the most appropriate strategy for addressing pest and disease problems. The *Musa* breeding program of the International Institute of Tropical Agriculture (IITA), in collaboration with NARO, aims at developing improved *Musa* genotypes, preferably triploids, with resistance to multiple pests and diseases, high and stable yield, improved agronomic traits and acceptable fruit quality. This usually involves crossing triploid cultivars with fertile diploids to produce tetraploids that generally display greater male and female fertility. Selected tetraploids are then crossed with improved diploids to produce sterile secondary triploids.

The objective of this study was to identify sources of resistance to *R. similis* in existing *Musa* germplasm and newly bred hybrids. This study also looked at the variability in reproductive fitness and virulence of different *R. similis* populations from Uganda and aimed to better understand host plant resistance to *R. similis* through genetic analysis of a segregating banana population and preliminary studies on the mechanisms of resistance.

In the first part of this research, a new method was developed for screening *Musa* germplasm for resistance to *R. similis*. This method is based on the inoculation of individual roots with a small number of *R. similis* females. The individual root screening method has several advantages compared to the standard greenhouse screening method. Fewer plants and a lower nematode inoculum are needed. By using individual roots, the evaluation of the host response to nematode infection is not influenced by differences in root growth rates among *Musa* genotypes. Moreover, primary roots of the same age can be selected for inoculation, avoiding bias caused by differences in host response to *R. similis* related to root age. In addition, this method seems to be able to pick up both constitutive and induced resistance.

In the second part of this research, this new screening method was used to evaluate available *Musa* germplasm and newly developed hybrids for resistance to *R. similis*. East African highland bananas are susceptible to *R. similis*. Tetraploid hybrids which are resistant to *R. similis* have been developed by crossing susceptible East African highland bananas with the resistant wild diploid Calcutta 4. This diploid has been widely used in *Musa* breeding programs as a male parent. Resistance was also identified in several diploid hybrids, which were used to further improve the tetraploid hybrids. TMB2× 9128-3 is the most resistant diploid...
identified so far and is often used as a parent in the breeding program. Tetraploid hybrids have been further crossed with improved diploids, resulting in secondary triploids. To date, five secondary triploids that are resistant to *R. similis* and seven that are partially resistant have been identified. Three of the secondary triploids with partial resistance to *R. similis* have good bunch characteristics and a matooke-like taste, while one resistant hybrid is recommended for juice production. In addition, new sources of resistance to *R. similis* have been identified, mainly among germplasm from Papua New Guinea.

In the third part of this research, different populations of *R. similis* from Uganda were compared for their variability in reproductive fitness and virulence. Four *R. similis* populations of different locations within Uganda (Namulonge, Mbarara, Ikuwe and Mukono) were collected and cultured monoxenically on carrot discs. The reproductive fitness of these four *R. similis* populations was compared on carrot discs as a function of time and inoculum level. These *in vitro* experiments showed that the *R. similis* population from Mbarara had the highest reproduction ratio. This was shown by comparing the final nematode population densities and by calculating growth curves using the Gompertz equation. The population from Mukono had the lowest reproduction ratio.

Pathogenicity experiments on host plants were carried out in pot trials. Both the final nematode population densities and percentages root necrosis on different host plants were higher for the *R. similis* population from Mbarara than for the populations from Namulonge, Ikuwe and Mukono. The *R. similis* population from Mbarara managed to break the resistance of ‘Pisang jari buaya’, known to be resistant to *R. similis*. The diploid hybrid TMB2x 9128-3 and ‘Yangambi km 5’ showed resistance against the four *R. similis* populations. These results indicate that differences in pathogenicity among different *R. similis* populations exist and should be taken into consideration in a breeding program. It is recommended to use the *R. similis* population from Mbarara in routine screening for identification of resistance in *Musa* germplasm. The fact that the *R. similis* population from Mbarara is more pathogenic than the other populations may have serious implications for farmers as Mbarara is the main banana growing area in Uganda. Recent observations in farmers’ fields in Mbarara show that *R. similis* infestation is localized for the moment but these farms are heavily infested and the incidence of toppling is high.

In the fourth part of this research, we studied the genetic analysis of segregation for resistance to *R. similis* in a diploid banana hybrid population. This diploid banana hybrid population was derived by crossing the diploid hybrids TMB2x 6142-1 and TMB2x 8075-7. The female parent TMB2x 6142-1 is susceptible to *R. similis* and derived from the cross between the East African highland banana ‘Nyamwihogora’ (AAA) and the wild banana ‘Long tavoy’ (AA), which are both susceptible to *R. similis*. The male parent TMB2x 8075-7 is resistant to *R. similis* and derived from the cross between the bred hybrid SH-3362 (AA) and the wild banana Calcutta 4 (AA), which are both resistant to *R. similis*. The diploid banana hybrid population was evaluated with the *in vitro* individual root inoculation method using the *R. similis* population from Namulonge. Of the 81 hybrids evaluated, 37 hybrids were resistant, 13 were partially resistant and 31 were susceptible to *R. similis*. A chi-square analysis indicated that resistance to *R. similis* is controlled by two dominant genes, A and B, both with additive and interactive effects, whereby recessive *bb* suppresses dominant A (either *A* or *B* required for partial resistance, both *A*- and *B*- confer full resistance, but *bb* suppresses *A*).

In the last part of this research, potential mechanisms of resistance to *R. similis* were studied. Knowledge on the mechanisms of nematode resistance may help the breeder to select for a desired characteristic for the breeding program, and it may also assist in the identification of resistance markers to facilitate screening of *Musa* germplasm. In a first set of experiments, the attraction and penetration ability of *R. similis* was compared among resistant and susceptible *Musa* cultivars. No significant differences in attraction and penetration of *R. similis* were observed between resistant and susceptible cultivars. Similar invasion rates of *R. similis* on resistant and susceptible *Musa* cultivars, suggests that the resistance is not due to physical
or mechanical barriers. Subsequently, histochemical experiments were carried out to detect whether there are differences in lignin and phenolic compounds between susceptible and resistant *Musa* cultivars. After *R. similis* infection, a higher number of phenolic cells were observed in the resistant cultivars compared to the susceptible cultivars. Phenolic cells were also observed in healthy plants, though the number was lower compared to nematode infected plants. It is assumed that preformed phenolic compounds in healthy roots do not contribute to the constitutive resistance of banana to *R. similis*. Lignification of the endodermis was observed in the susceptible cultivars early in time. Lignification in the resistant cultivars was only observed after 12 weeks. No lignified cells were observed in the cortex of any cultivar. More detailed studies are needed to understand the role of phenolic compounds and lignin formation in relation to host plant response to nematode infection.

Random amplified polymorphic DNA analysis of 28 clones

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The genetic diversity of 28 banana clones belonging to different genomic groups (AAA, AAB, ABB, AA, AB and BB) and ploidy groups (triploids and diploids) was evaluated based on morphological traits and random amplified polymorphic DNA (RAPD) profiles was evaluated. The bananas were collected from the Banana Research Station in Kannara, the Banana Nursery Farm in Thiruvananthapuram, and the College of Agriculture in Vellayani. Out of the 41 decamer primers screened for RAPD analysis, 34 produced amplification. Of the 123 bands generated, 116 were polymorphic and 7 were monomorphic. Finally, 6 primers (OPA-01, OPA-03, OPA-13, OPB-04, OPB-10 and OPB-12) were used to analyse the 28 clones. These primers yielded 46 scorable bands with an average of 7.66 bands per primer. In the dendrogram, the 28 clones clustered into 14 groups at a distance of 0.20.

Eight out of 12 Nendran clones (AAB), ‘Chengazhikodan’, ‘Myndoli’, ‘Kalie-than’, ‘Vellayani nendran’, ‘Zanzibar’, ‘Mysore ethan’, ‘Manjeri nendran’ and ‘Changanasseri nendran’ formed a single cluster at a distance of 0.25 and Palayankodan clones (AAB) like, ‘PKNNR’, ‘Pisang Ceylon’, ‘Motta poovan’, ‘Chandra bale’ and ‘Palode palayankodan’ formed another cluster. ‘Red banana’ and ‘Vellakappa’ (AAA) as well as ‘Pisang Ceylon’ and ‘PKNNR’ (AAB) formed clusters at a distance of 0.12. ‘Kunnan’ and ‘Njali poovan’ (AB) formed a cluster at a distance of 0.22. ‘Kadali’ and ‘Pisang lilin’ (AA) as well as ‘Quintal’ and ‘Padalamurian’ (AAB) formed clusters at a distance of 0.24. The triploids ‘Attu nendran’ (AAB), ‘Monthan’, ‘Robusta’ (AAA), ‘Koonoor ethan’ (AAB), and ‘Vellapalayankodan’ (AAB) and the diploid ‘Ilavazha’ (BB) formed individual clusters and diverged the most.

Tocher’s method was used to analyse morphological characters. The cultivars formed 6 clusters. ‘PKNNR’, ‘Pisang Ceylon’, ‘Motta poovan’, ‘Chandra bale’ and ‘Palode palayankodan’ (Palayankodan, AAB), ‘Kadali’ and ‘Pisang lilin’ (AA), ‘Kunnan’ and ‘Njali poovan’ (AB) formed the first cluster. ‘Red banana’ ‘Vellakappa’ and ‘Robusta’ (AAA), ‘Myndoli’, ‘Mysore ethan’, ‘Changanasseri nendran’, ‘Kaliethan’, ‘Chengazhikodan’, ‘Manjeri nendran’, ‘Padalamurian’ and ‘Attu nendran’ (AAB), ‘Monthan’ and ‘Peyan’ (ABB), and Ilavazha (BB) formed the second cluster. ‘Vellayani nendran’ and ‘Zanzibar’ (AAB) formed the third cluster. ‘Quintal’ (AAB) formed the fourth cluster, ‘Vellapalayankodan’ (AAB) the fifth and ‘Koonoor ethan’ (AAB) the sixth.
Rediscovery of *Musa splendida* A. Chevalier and description of two new species (*Musa viridis* and *Musa lutea*)

An article by Ramon Valmayor, Le Dinh Danh and Markku Hakkinen published in the March 2004 issue of the Philippine Agricultural Scientist (Vol. 87(1):110-118) illustrates the distinguishing characteristics of the newly rediscovered *Musa splendida* and two recently described *Musa* species from Vietnam.

*Musa splendida*, known in Vietnam as ‘Chuoi gai’, is a very rare species of wild banana that was drifting toward oblivion. Cheesman, who revised the classification of bananas, did not include its description in his monumental series “Critical Notes on Species”. Simmonds doubted its status as a valid species and Champion associated it with *Musa sanguinea* and *Musa laterita*. The leading banana taxonomists of the world seemed were prepared to relegate *M. splendida* as *species ignota*.

A comprehensive report on the *Musa* germplasm resources of Vietnam listed banana cultivars, ornamental species and wild relatives but never mentioned *M. splendida*. An illustrated flora of Vietnam showed drawings of indigenous Musaceae, but *M. splendida* was not included. However, some elderly people in Vietnam maintained that ‘Chuoi gai’ existed between Lao Cai and Sa Pa. Inge Van den Bergh, currently an expert associate at the INIBAP regional office in the Philippines, surveyed the original home of *M. splendida* and discovered large populations still thriving in the Red River Valley, near Lao Cai. The suspicion that *M. splendida* was a mere synonym of either *M. sanguinea* or *M. laterita* was dismissed with the demonstration of their distinguishing characters.

Recent characterization studies of *Musa* accessions at the Phu Ho Fruit Research Center in Vietnam describe two new species, *Musa viridis* and *Musa lutea*. The former is known locally as ‘Chuoi rung hoa sen’ and the latter as ‘Chuoi rung hoa do’. The word ‘Chuoi’, meaning banana, is part of the actual name which is often descriptive. ‘Chuoi rung’ means jungle banana. ‘Chuoi rung hoa sen’ means jungle banana with lotus colored flower and ‘Chuoi rung hoa do’ means jungle banana with red flower.

The article also presents a diagnostic characterization of *M. viridis* and *M. lutea* to differentiate them from *Musa balbisiana*, *Musa acuminate* and *Musa itinerans* and local species such as *M. sanguinea*, *M. splendida* and *M. laterita*. Finally, *M. viridis* and *M. lutea* are differentiated from each other by the color of their fruits and male buds. The Latin terms *viridis* and *lutea* were selected to highlight the difference in colour of the immature fruits, which are silvery green in *M. viridis* and yellow in *M. lutea*.

Presence of banana bunchy top virus in Angola

Banana bunchy top disease, caused by the Banana bunchy top virus (BBTV), is one of the most serious diseases affecting banana worldwide (Dale 1987). Symptoms include dark green spots along the leaf veins, especially the midrib and petiole, upright leaves with wavy margins, stunted growth and leaves more erect than normal, giving the plant a rosetted, or ‘bunchy top’, appearance (Robinson 1996, Jones 2000).

Recently, symptoms of bunchy top were observed in the fields of small-scale farmers in Mabuia (48 masl: S09°01’, E013°41’) and Boa Esperanca (52 masl; S08°57’, E013°40’) in Bengo Province, South of Luanda, in Angola. The disease was found on plantains (False horn) and the Cavendish cultivar ‘Poyo’. It is known that cultivars in the Cavendish subgroup are highly susceptible to bunchy top (Thomas and Iskra-Caruana 2000).
is the first report of the disease in Angola. Countries in Africa where BBTV has been reported include Burundi, Central African Republic, Congo, Democratic Republic of Congo (DRC), Egypt, Gabon, Malawi and Rwanda (Thomas and Iskra-Caruana 2000). Angola and the DRC share common borders and it is likely that infected planting materials were exchanged. BBTV is transmitted through conventional planting material such as corms, corm pieces that have a growing point and suckers. The vector of BBTV is the banana aphid.

Destroying the infected material is the main way of controlling the disease and the method advocated in Angola.

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Containing banana xanthomonas wilt

A workshop on “Developing a coherent regional response to the banana xanthomonas wilt epidemic in East and Central Africa”, was held in Kampala, Uganda, on 14-18 February 2005. Organized with the support of the Food and Agriculture Organization of the United Nations (FAO), the International Development Research Centre of Canada (IDRC) and the International Network for the Improvement of Banana and Plantain (INIBAP), it brought together regional and national stakeholders from Uganda, Ethiopia, the Democratic Republic of Congo, Kenya, Tanzania and Rwanda, as well as international specialists. The participants considered priority needs for research, outreach and policy activities to address this regional threat and identified and agreed on the following key issues for short- to medium-term action.

- In advance of the epidemic: to track its spread and prepare for its arrival.
- At the advancing disease front: to slow its advance and mitigate its impact.
- In areas where the disease had become established: to rebuild production systems and improve livelihoods.

- Throughout the region: to coordinate and monitor efforts, exchange information and facilitate policy dialogue.
- The technical core of the disease management campaign, which needs to be developed and implemented using farmer participatory approaches, will involve:
  - Debudding (removal of male bud) and field sanitation, reinforced by:
    - statutory measures at national and local levels to slow the spread of disease (quarantine regulations) and containment and control measures (by-laws);
    - awareness raising efforts at the international, regional, national and local levels, directed towards decision-makers and the general public, in order to mobilize the necessary resources and ensure support for the campaign;
    - improved ‘seed’ systems for supplying clean, high quality planting material; and
    - improved agronomic practices to increase productivity and sustainability, combined with the dissemination of utilization options to improve livelihoods.
• Evaluation and introduction of new varieties which are acceptable to farmers and consumers and are more tolerant to BXW.

Other actions needed include:
• research to ensure the soundness of the current control measures and to generate new options for the future;
• mechanisms for planning, information exchange and coordination at the national and regional level to ensure the most cost-effective use of resources;
• and evaluating the impact of the activities to inform policy and strengthen strategies for managing trans-boundary pests and diseases.

Given the scale of the problem and the seriousness of the threat to livelihoods, the participants invited national governments and donors to invest in the proposed framework “as offering the best strategy for containing the spread of the disease, mitigating its immediate effects on livelihoods and eventually restoring the productivity and sustainability of banana-based production systems”.

For more information:
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Controlling banana xanthomonas wilt through debudding

Banana xanthomonas wilt, which is caused by the bacterium *Xanthomonas campestris* pv. *musacearum*, was first observed in Uganda in 2001 and has since spread to 31 out of 56 districts. Technologies used to combat similar bacterial wilt diseases in Latin America and Asia have been introduced to Ugandan farmers and campaigns have been conducted to raise awareness of the disease and ways to control it. Since the bacteria are believed to be transmitted by insects and contaminated farm tools, the removal of diseased plants, early debudding and the use of clean farm tools could thus significantly reduce new infections. Small-scale Ugandan farmers have started to apply these techniques and have been able to prevent new infections or have even eradicated the disease from their farms.

Fredrick Kisegerwa has a field of ‘Pisang awak’ (ABB) in Central Uganda. He started debudding his banana plants in January 2004 after observing a strange disease on several of his plants. He got the information about the disease and its control measures from the sub-county agricultural extension officer and had heard about it on the radio. At that time there were about 20 sick plants on his farm. He rouged these plants by digging them up, chopping them into pieces and burying them. In the year after he started debudding, which he does twice a week, only four of his plants contracted the disease, three of them in the first two months after he started debudding. At first, he used a knife attached to a stick but he was advised to use a forked stick (Figure 1) to avoid spreading the disease through contaminated knives. He also says that he sees fewer insects, probably because the male buds have been removed.

Some Ugandan farmers participating in a banana germplasm evaluation and multiplication project, funded by the Common Fund for Commodities and managed by the Uganda National Agricultural Programme and INIBAP, started debudding their plants when the disease closed in on their fields. Their experience shows that prevention – debudding combined with controlling sources of infection – can protect the plants even when the surrounding fields are heavily infected. This is especially important in sizeable plantations where the eradication of a large number of sick plants is costly and labour-intensive.

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Survey on banana bract mosaic disease in Kerala

A survey on the incidence of banana bract mosaic disease (BBrMD), locally known as Kokkan disease, was carried out in 50 randomly selected farms in Kalliyoor Panchayat, southern Kerala, India. Ten plants from each site were sampled. The cultivars sampled were ‘Nendran’ (AAB), ‘Red banana’ (AAA), ‘Robusta’ (AAA), ‘Palayamkodan’ (AAB) and ‘Rasakadali’ (AB).

BBrMD was first reported on ‘Nendran’ in Kerala by Samraj et al. (1966). The first symptoms appear on the leaf sheath as longitudinal, irregular, reddish or pinkish streaks of varying sizes (Estelitta et al. 1996). Later on, the pseudostem becomes abnormally red in colour and spongy in texture (Estelitta et al. 1996). The symptoms were scored using the following scale:

0 = No symptom
1 = Reddish brown streaks over 1-25% of the pseudostem
2 = Reddish brown streaks over 26-50% of the pseudostem
3 = Reddish brown streaks over 51-75% of the pseudostem
4 = Reddish brown streaks over more than 75% of the pseudostem

The plants were observed at weekly intervals and symptoms appeared three to four months after planting. Over 130 plants scored 1, more than 160 scored 2, nearly 100 scored 3 and less than 70 scored 4. The incidence of disease appeared greater on ‘Red banana’.

References


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The story behind the name ‘Yangambi km 5’

‘Yangambi km 5’ (AAA) has numerous, small fruits that have a very pleasant taste when ripe. It is a vigorous plant that remains productive on poor soils and which has become well-known for being resistant to black leaf streak disease, caused by Mycosphaerella fijiensis.

Its somewhat peculiar name has recently caused people to speculate about its meaning and origin. It just happens that in the 1950s I was in charge of the banana programme at Yangambi and may be able to shed some light on the matter.

The name comes from the location of the fruit gardens at the “Institut National pour les Études Agronomiques au Congo Belge” (INEAC), which is now called INERA. The research divisions, with their laboratories and experimental fields, were built along two main road axes. The original axis followed the northern bank of the Congo River, while the second axis was perpendicular and stretched northwards for about 25 km. The main crop divisions (oil palm, rubber, coffee, etc.) were clustered around the fifth kilometre of this axis, with the consequence that the banana field, with its ‘Gros Michel’, Silk, Prata, Cavendish and this mysterious cultivar, were located at ‘km 5’.

So why ‘Yangambi km 5’ and not a more convenient cultivar name? Simply because we were not able to identify the plant. It did not fit any of the descriptions and illustrations available at the time. For a while we thought it was a diploid, but chromosome counting at the division of plant genetics showed that ‘Yangambi km 5’ was a triploid.

After a visit to Yangambi, in 1957 if I remember well, the regretted Jean Champion introduced ‘Yangambi km 5’ in Guinée’s
local scientists have classified numerous cultivars from Southeast Asia. In Thailand, Dr. Silayoi identified a ‘Kluai thong ruang’, the description of which strongly reminds me of ‘Yangambi km 5’ except for its deciduous fruits, but the most serious candidate for synonymy with ‘Yangambi km 5’ is ‘Kluai hom bao’ from southern Thailand. Other possible synonyms in Thailand are ‘Kluai nam nom’, ‘Kluai chak nuan’ and ‘Kluai hom hak’. It is possible that ‘Yangambi km 5’ was brought from Thailand directly to Congo by a miner working in one of the many mines in the Kilo-Moto area.

We may have to live with the name ‘Yangambi km 5’ for a while still, unless molecular tools establish that it is a Thai cultivar, in which case the Thai appellation would be preferable.

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**Announcements**

**Conference on black leaf streak disease**

An international congress on the management of black leaf streak disease in Latin America and the Caribbean will be held in San Jose, Costa Rica, 1-2 November 2005. The conference is organized by CORBANA, INIBAP and MUSALAC. The four sessions will be devoted to the impact of the disease on production and quality; the epidemiology, biology and ecology of the fungus; chemical control; and biological control and genetic improvement. For more information consult the INIBAP website at www.inibap.org.

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**Symposium on plant biotechnology**

The VII International Symposium of Plant Biotechnology will be held 17-21 April 2006 at the Institute of Plant Biotechnology, Villa Clara, Cuba. Among the themes covered will be genetic transformation, bioinformatics, tissue culture, genetic improvement, biosafety and intellectual property rights. A workshop will be devoted to bananas and plantains. For more information, contact Orlando Gregorio Chaviano at biotec2006@ibp.edu.cu.
Instructions to authors

**InFoMUSA** is an international journal published twice a year in English, French and Spanish. Our focus is to provide an outlet for research results and reports of interest to the *Musa* community. As **InFoMUSA** publishes articles on any *Musa*-related issue, authors should aim for simple and clear phrases that avoid unnecessary jargon in order to make their paper accessible to readers in other disciplines.

Manuscripts should be prepared in English, French or Spanish and should not exceed 2500 words, including references. 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.

Manuscripts can be sent as e-mail attachments or put on a 3.5-inch disk for PC-compatible machines. Please indicate the name and version of the word processing software used and the author’s e-mail address. In either case, we will need to receive by mail two printed copies of the manuscript.

**Title**: The title should be as short as possible and should not have numbers, acronyms, abbreviations or punctuation.

**Abstract**: An abstract, not exceeding 200-250 words, should be provided. It should concisely summarise the basic contents and should be sent in the same language as the manuscript. Translations (including the title) into the two other languages should also be sent if this is possible.

**Key words**: Provide a maximum of six key words, in alphabetical order, below the native-language abstract.

**Introduction**: The introduction should provide the rationale for the research and any relevant background information. Since it is not meant to be an exhaustive review of the topic, the number of references should be kept to a minimum. Introductions on the importance of bananas as a staple food or a traded commodity should be avoided, unless they are absolutely necessary for the comprehension of the article.

**Materials and methods**: The authors should provide enough details of their experimental design to allow the reader to gauge the validity of the research. For commonly used materials and methods, a simple reference is sufficient.

**Results**: The unit should be separated from the number by a single space and follow SI nomenclature, or the nomenclature common to a particular field. Unusual units or abbreviations should be defined.

Present data in the text, or as a figure, or a table, but never in more than one of these ways. Avoid extensive use of graphs to present data that could be more concisely presented in the text or in a table. Limit photographs to those that are absolutely necessary to show the experimental findings.

**Discussion**: The discussion should not contain extensive repetition of the results section nor should it reiterate the introduction. It can be combined with the results section.

**References**: All references to the literature made in the text should be referred to by author(s) and year of publication (e.g.: Sarah *et al*. 1992. Rowe 1995). References to not widely circulated documents, such as annual reports, and citations of personal communications and of unpublished data should be avoided. A list of references, in alphabetical order, should be provided at the end of the text.

Please follow the style shown below:


**Illustrations and tables**: These should be numbered consecutively and referred to by these numbers in the text. Each illustration and table should include a clear and simple caption. Figures and tables should be inserted after the references or in separate files.

**Graphs**: Provide the corresponding raw data with the graphs, if possible in Excel format.

**Drawings**: Provide originals if this is possible.

**Photographs**: We prefer hard-copy printouts of photographs (bright paper with good contrast for black and white photographs; good quality proofs and films or original slides for colour photographs), but please remember that we will not return them. We will publish pictures that have been scanned or taken with a digital camera as long as the resolution is high enough (1 million pixels or a minimum of 300 dpi when the photograph is in real size). Acceptable file types are JPEG, TIFF and EPS. Avoid sending photos inserted in a Word or Power Point document, unless they are accompanied by a better quality alternative.

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

**Cultivar names**: The name of the cultivar should be placed between single quotation marks. If the name is a compound noun, only the first word starts with a capital letter, unless the other refers to a place or person. Use the most commonly agreed upon name, such as ‘Grande naine’ and avoid local variations or translations, such as ‘Gran Enano’.

**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.
In press


Recent publications


S. Mohan Jain and R. Swennen (eds). 2004. Banana improvement, cellular, molecular biology, and induced mutations. This 392-page book, co-published by FAO/IAEA and INIBAP, presents the results from the FAO/IAEA Coordinated Research Project entitled “Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes”. The book also contains several review papers providing up-to-date information on biotechnological tools that can be used to produce new Musa varieties with desirable characters in a more rapid and efficient way.

To obtain a complete list of our publications, consult our website or contact Leila Errachiq at INIBAP headquarters in Montpellier.
E-mail: l.errachiq@cgiar.org