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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. The Programme has four specific objectives:

• To organize and coordinate a global research effort on banana and plantain, aimed at the development, evaluation and dissemination of improved cultivars and at the conservation and use of Musa diversity
• To promote and strengthen collaboration and partnerships in banana-related research activities at the national, regional and global levels
• To strengthen the ability of NARS to conduct research and development activities on bananas and plantains
• To coordinate, facilitate and support the production, collection and exchange of information and documentation related to banana and plantain.

INIBAP is a programme of the International Plant Genetic Resources Institute (IPGRI), a Future Harvest center.
Integrated crop management strategies for plantain production and control of black leaf streak (black Sigatoka) disease in the Democratic Republic of Congo

P. Mobambo Kitume Ngongo

Plantain (Musa spp., AAB group) is an important staple food in many countries of the humid tropics. It is among the most important carbohydrate sources in the diet of people in these regions. Its low labour requirement and relatively high-energy output make plantain a suitable staple for areas where labour shortage is usually the main constraint to production. The crop is mainly grown by small-scale farmers and it is an integral component of most farming systems in West and Central Africa, where about 50% of the world’s plantain is produced (Wilson 1987, FAO 1990).

In spite of its importance to local people, plantain has long been ignored by agricultural researchers in the region, since it had no major disease problems until the 1970s and was therefore regarded as a disease-free crop in Africa (Wilson 1987). Twenty-five years ago, however, the crop was threatened by black leaf streak (black Sigatoka), an air-borne leaf spot disease caused by the fungus Mycosphaerella fijiensis Morelet. The disease spread rapidly into all plantain-producing regions of Africa. Black Sigatoka is the most destructive leaf disease of plantain, as it is spreading inexorably to all major lowland plantain-growing regions as the dominant leaf spot (Meredith and Lawrence 1970). Plantain yield loss of 76% due to black Sigatoka has been reported during the second cropping cycle, while the whole complex of diseases, pests and soil fertility decline together reduced yield by 93% (Mobambo et al. 1996a). As a perennial starchy crop, plantain requires a considerable time to mature, resulting in longer exposure to diseases, pests and in depletion of soil nutrients.

The soil-disease-pest complex can be controlled by the combination of inorganic fertilizers, fungicides and insecticides/nematicides. In Africa however, chemical control strategies are socio-economically and environmentally unsound in the framework of the resource-poor smallholders growing plantain. Chemicals are very expensive and their applications may be hazardous to health in the village homesteads where the bulk of plantain is grown. Therefore, proper soil management using several crop residues mulches to improve the organic matter and nutrient content of the soil could reduce the soil-disease-pest complex effects on plantain with low inputs.

The objectives of the research reported here were to compare field performance and yield of plantain under different practices of soil fertility management and disease control.

Materials and methods

Location of the experiment
Investigations were carried out at Kinshasa (4°22’S, 15°21’E, western Congo), which is at 390 m above sea level (Anonymous 1985). The soil of the experimental site is a latosol derived from deposited sands, well drained, but poor in nutrients and highly acidic. Annual rainfall averages 1800 mm and average temperature is 24.5°C.

Plant materials and treatments
Musa AAB cv. ‘Yumba’, locally widespread, was used in this experiment. Planting materials still constitute a constraint for plantain production in rural areas. As it is impossible to get many and uniform plantain suckers at once, the investigation started by vegetative multiplication of planting materials (technique described by Auboiron 1997) in order to obtain 625 plants for the experiment: 5 treatments x 5 replications x 25 plants per treatment.

Plantain corm stumps were split into sets of 50 each and treated with wood ashes. They were air-dried for 24 hours before being planted in 15 cm-diameter plastic bags almost filled with forest top-soil. New sprouts emerged after 4 weeks from the date of planting and up to 20 new plants were obtained from a corm.

Plants were grown in half-shade conditions and watered regularly. They were transplanted in the field 3 months later, when they had 3-4 true leaves.

Four different treatments to prevent infection by microorganisms, based on cultural practices, were compared: crop residues mulches (wood sawdust or rice husk), cover crop (Vigna unguiculata) and fertilizers (NPK). Non-treated plants were used as control.

Field layout and cultural practices
The experimental design was a randomized complete block with five plots-treatments and five replications. The plot size was 15 m x 10 m with 25 plants spaced by 3 x 2 m, resulting in a plant density of 1667 per ha. Data were recorded only on the nine central competitive plants.

Every 3 months, crop residues mulches were applied to the soil around the stem in mulched plots using one head-pan (10 kg). In fertilized plots, 300 kg N, 60 kg P₂O₅ and 550 kg K₂O per ha per year were split into six applications during the rainy season: urea at a rate of 65 g per plant per application, phosphorus at a rate of 20 g per plant per application, and nitrate of potash at a rate of 89 g per plant per application.

For each treatment, soil samples were taken at about 50% flowering stage using a soil hand auger up to 20 cm depth, where plantain has the majority of its roots (Swennen 1984, Purseglove 1988). These samples were air-dried in the laboratory, crushed, passed through 0.5 and 2 mm sieves and analyzed.

Evaluation of host response to black Sigatoka, growth and yield parameters
The disease development was evaluated every week using the “symptom evolution time”, which is the number of days between the appearance of symptoms of stage 1 of the disease development (Fouré 1982) assimilated to stage b of the cigar (Brun 1963) and the appearance of spots with dry centres (stage 6 of the disease, Fouré 1982, 1987). The “youngest
leaf spotted” which is the leaf with 10 or more discrete necrotic lesions with dry centres (Meredith and Lawrence 1970, Foure 1982, 1987) and the “life time of the leaf”, which is the number of days between the cigar-stage b of the leaf and leaf death (100% leaf area necrotic), either due to senescence or black Sigatoka (Mobambo et al. 1994) were also recorded.

Disease severity was evaluated every two weeks, from two months after planting until flowering. The percentage of leaf area with symptoms was recorded using the modified scale of Stover and Dickson (1970) as described at the International Institute of Tropical Agriculture (Mobambo et al. 1993a).

Growth parameters evaluated include height of pseudostem, girth of pseudostem, number of leaves emerged and height of the tallest sucker. They were recorded on each plant from 2 months after planting until flowering as described by Swennen and De Langhe (1985).

Yield parameters evaluated were number of hands per bunch, number of fruits per bunch and bunch weight.

Data collected were analyzed using the ANOVA procedures of Statistical Analysis System (SAS 1988) for randomized complete block design. The Duncan Multiple Range (DMR) test at the 0.05 significance level was used to compare treatment means for each parameter.

Results and discussion

Soil conditions

Soil analysis results presented in Table 1 showed significant differences in the amounts of nutrients between crop residues mulches (wood sawdust and rice husk) and other management practices, such as cover crop (Vigna unguiculata) and mineral fertilizer (NPK). Meanwhile, statistical differences were found between rice husk and wood sawdust, with rice husk as the best improving soil fertility level. According to the scales of Black (1965) and Brady (1984), in plots mulched with crop residues the soil was in general moderately acidic with very high organic carbon, high total nitrogen, moderate calcium, moderate magnesium and high potassium. In the non-mulched plantain plots however, the soil was in general moderately acidic with very low organic carbon, moderately low total nitrogen, low calcium, very low magnesium and very low potassium.

These results indicate that the amounts of soil nutrients are higher in the mulched plots than in the non-mulched plots. Crop residues mulches constitute better sources of nutrients and act therefore as a fertilizer. As pointed out by Lal and Kang (1982), organic matter constitutes a key component of soil fertility, as a reservoir of nutrients, as a main source of cation exchange capacity and as major promoter of aggregate structural stability.

Table 1. Selected soil chemical properties under different plantain management practices at Kinshasa, western Congo, 1998.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Organic Carbon (%)</th>
<th>Total Nitrogen (%)</th>
<th>Exchangeable cations (meq/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>Control</td>
<td>4.2 a</td>
<td>1.15 a</td>
<td>0.11 a</td>
<td>1.22 a</td>
</tr>
<tr>
<td>Wood sawdust</td>
<td>6.2 c</td>
<td>3.51 c</td>
<td>0.25 b</td>
<td>6.51 c</td>
</tr>
<tr>
<td>Rice husk</td>
<td>6.8 d</td>
<td>3.79 d</td>
<td>0.28 b</td>
<td>7.52 d</td>
</tr>
<tr>
<td>Vigna unguiculata</td>
<td>5.2 b</td>
<td>2.15 b</td>
<td>0.22 b</td>
<td>4.07 b</td>
</tr>
<tr>
<td>N-P-K</td>
<td>5.6 b</td>
<td>2.23 b</td>
<td>0.26 b</td>
<td>5.63 c</td>
</tr>
</tbody>
</table>

Within columns, means followed by the same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.

With respect to the youngest leaf spotted (YLS), results show the same trend as for the symptom evolution time (Table 2). There were significant differences between the plantain mulched with crop residues and the non-mulched plantain. On mulched plantain, YLS was 11 for rice husk and 9 for wood sawdust, whereas on both the fertilizer-treated plantain and on the cover-cropped plantain the YLS was 8. The non-treated plantain (control) had the lowest YLS value, 6.

These results indicate that when using the rice husk mulch the plant gains three healthy leaves comparing with the fertilizers or cover crop treatments, and five healthy leaves against the control. Therefore, with a leaf emergence time of about one per week for plantain in general, the soil fertility (Table 1) due to rice husk mulch slowed the symptom evolution by 3 and 5 weeks compared respectively to that of the fertilized and cover-cropped plots and the control.

Table 2. Host-plant response to black Sigatoka of plantain under different management practices at Kinshasa, western Congo, 1998.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Symptom evolution time (SET, days)</th>
<th>Youngest leaf spotted (YLS)</th>
<th>% Leaf area with symptom (% LAWS)</th>
<th>Life time of leaf (LTL, days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.0 a</td>
<td>5.5 a</td>
<td>19.6 d</td>
<td>62.5 a</td>
</tr>
<tr>
<td>Wood sawdust</td>
<td>50.0 c</td>
<td>9.3 c</td>
<td>4.2 a</td>
<td>125.7 d</td>
</tr>
<tr>
<td>Rice husk</td>
<td>56.8 c</td>
<td>10.9 d</td>
<td>3.8 a</td>
<td>130.3 d</td>
</tr>
<tr>
<td>Vigna unguiculata</td>
<td>35.5 b</td>
<td>7.5 b</td>
<td>10.3 c</td>
<td>80.3 b</td>
</tr>
<tr>
<td>N-P-K</td>
<td>40.0 b</td>
<td>8.1 b</td>
<td>6.9 b</td>
<td>103.3 c</td>
</tr>
</tbody>
</table>

Within columns, means followed by the same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.
Regarding leaf life time, significant differences were also found between plants mulched with crop residues and those that were not mulched (Table 2). Slower disease development on mulched plantain prolonged the lifetime of leaves. In plantain treated with rice husk and wood sawdust, black Sigatoka needed almost 9, 7 and 4 weeks longer to destroy the leaves as compared respectively to the control, the cover-cropped and the fertilized plantain. The control plantain was the most affected by the disease. As already reported, all plantain cultivars (Musa spp., AAB group) over the world are susceptible to black Sigatoka (Fouré 1987, Mobambo et al. 1996b).

The difference in the host response to black Sigatoka between the plantain mulched with crop residues and non-mulched plantain is mainly attributed to the difference in soil fertility. The higher the soil fertility level, the lower the black Sigatoka severity. On better soils this is expressed in a slower symptom development; older leaves bearing dry spots, less leaf area with black Sigatoka symptoms and longer life time of leaves (Mobambo et al. 1994).

### Growth and yield performances

Results presented in Table 3 show significant differences for all the parameters studied: plant height (PH), plant girth (PG), number of emerged leaves (NEL), days to flowering (DF), days for fruit filling (DFF), days to harvest (DH) and height of the tallest sucker (HTS).

For all treatments (crop residues mulches, fertilizer or cover crop) the plants had a similar height, whereas they were shorter than the control. However, regarding plant girth and number of emerged leaves, the plantain mulched with crop residues performed better than the non-mulched plantain. Bigger plant girth and lower number of leaves were obtained on plants treated with rice husk and wood sawdust than on the non-mulched plants. Plants mulched with rice husk flowered significantly earlier and had a longer fruit-filling period than those under other treatments. They flowered 5 months earlier than the control and about 1 to 2 months earlier than the fertilized and cover-cropped plantains. The combined effect resulted in a shorter production cycle for the plantain mulched with rice husk, whose bunches were harvested 104 and 28 days earlier than in the control and fertilized plantain respectively. Plantain mulched with rice husk was harvested 16 days earlier than that mulched with wood sawdust. It also showed better suckering, the tallest sucker, i.e. the sucker that will continue as the next production cycle, being significantly taller than for other treatments. This should normally result in a shorter ratoon cycle for the plantain treated with rice husk as compared to other treatments.

The yield components evaluated were the number of hands per bunch, number of fruits per bunch and bunch weight (Table 4). There were significant differences between the plantain mulched with crop residues (wood sawdust and rice husk) and the non-mulched plantain (control, cover crop and fertilizer) regarding the number of hands per bunch and number of fruits per bunch (Table 4). The mulched plantain had a higher number of hands and fruits per bunch than the non-mulched.

Yield per hectare was calculated from the average bunch weight multiplied by plant density. Yield was significantly different between the plantain mulched with rice husk and other treatments. The yield of the best-performing plantain treated with rice husk was 46%, 37% and 26% higher than those of control, cover-cropped and fertilized plantain, respectively. The yield of plantain mulched with rice husk was 14% higher than that obtained with wood sawdust. These results indicate that crop residues mulches confer important advantages to plantain cultivation: higher yield, earlier maturity or shorter production cycle and bigger girth allowing reduction of losses from wind-damage, another important constraint to plantain production (Mobambo et al. 1996a).

### Conclusion

The research reported here compares different management practices of plantain production. The effects of the crop residues mulches (wood sawdust and rice husk) were compared to those of fertilizer application and cover crop for soil fertility, black Sigatoka severity, growth and yield parameters of plantain. For all parameters evaluated, the plantain mulched with crop residues performed better than the non-mulched plantain. Soil fertility is the critical factor responsible for the difference between crop residues mulches, cover crop and fertilizer. Because of the high level of fertility due to the application of crop residues mulches, plantain was less affected by black Sigatoka and consequently better growing than when receiving no mulch. Among crop residues mulches, rice husk was statistically better than wood sawdust.

### Table 3. Growth parameters of plantain under different management practices at Kinshasa, western Congo, 1998.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PH (cm)</th>
<th>PG (cm)</th>
<th>NEL</th>
<th>DF</th>
<th>DFF</th>
<th>DH</th>
<th>HTS at harvest (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>360.3 b</td>
<td>65.4 a</td>
<td>44 d</td>
<td>370 d</td>
<td>76 a</td>
<td>446 d</td>
<td>78.0 a</td>
</tr>
<tr>
<td>Wood sawdust</td>
<td>345.5 a</td>
<td>74.6 b</td>
<td>35 a</td>
<td>255 b</td>
<td>103 c</td>
<td>358 b</td>
<td>105.0 b</td>
</tr>
<tr>
<td>Rice husk</td>
<td>340.0 a</td>
<td>76.6 b</td>
<td>34 a</td>
<td>232 a</td>
<td>110 d</td>
<td>342 a</td>
<td>145.0 c</td>
</tr>
<tr>
<td>Vigna unguiculata</td>
<td>349.5 a</td>
<td>68.8 a</td>
<td>41 c</td>
<td>295 c</td>
<td>86 b</td>
<td>381 c</td>
<td>80.5 a</td>
</tr>
<tr>
<td>N-P-K</td>
<td>342.2 a</td>
<td>66.8 a</td>
<td>38 b</td>
<td>268 b</td>
<td>102 c</td>
<td>370 bc</td>
<td>86.8 a</td>
</tr>
</tbody>
</table>

Within columns, means followed by the same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.

### Table 4. Yield parameters of plantain under different management practices at Kinshasa, western Congo, 1998.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of hands per bunch</th>
<th>No. of fruits per bunch</th>
<th>Bunch weight (kg)</th>
<th>Yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.0 a</td>
<td>75 a</td>
<td>9.5 a</td>
<td>15.8 a</td>
</tr>
<tr>
<td>Wood sawdust</td>
<td>6.5 b</td>
<td>88 c</td>
<td>15.0 c</td>
<td>25.0 c</td>
</tr>
<tr>
<td>Rice husk</td>
<td>6.5 b</td>
<td>90 c</td>
<td>17.5 d</td>
<td>29.2 d</td>
</tr>
<tr>
<td>Vigna unguiculata</td>
<td>6.2 a</td>
<td>82 b</td>
<td>11.0 a</td>
<td>18.1 a</td>
</tr>
<tr>
<td>N-P-K</td>
<td>6.2 a</td>
<td>87 bc</td>
<td>13.0 b</td>
<td>21.7 b</td>
</tr>
</tbody>
</table>

Within columns, means followed by the same letter are not significantly different at the 0.05 probability level, according to the Duncan Multiple Range test.
Therefore, proper management of organic matter is essential for the sustainable productivity of plantain, by minimizing the black Sigatoka severity with low inputs. Since plantain is grown mainly by small-scale farmers in Africa, chemical fertilizers are not readily and economically available. Thus, the potential of traditional organic fertilizers such as compost, farmyard manure and crop residues mulches need to be better exploited. A study integrating organic resources and soil fauna may help to understand the mechanisms regulating the biological processes for the improvement of soil fertility in relation with the sustainability of plantain production, disease and pest severity.

Acknowledgements
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References

Diseases

Distribution of black Sigatoka

The spread of black Sigatoka throughout Venezuela, 1997-2000


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The work presented here aims to describe the current situation of black Sigatoka in Venezuela, the course of its spread, its relationships with various climatic factors which determine its aggressiveness and measures taken for its control. For this purpose, an expedition was made into different areas in the south-east of Venezuela, collecting samples showing typical symptoms of the disease for identification, questioning growers and analyzing weather data (relative humidity, rainfall and temperature).

**Current situation and spread of the disease**

Black Sigatoka was detected for the first time in Venezuela in 1991 in the state of Zulia in the western region (Haddad et al. 1992, Escobar and Ramirez 1995), and then spread into various zones and states (Martínez 1997, Martínez et al. 1998). Mention is made in this report of its arrival in the state of Bolivar and, between 1999 and 2000, in the states of Delta Amacuro and Amazonas, in the extreme east and south of the country, respectively. On the basis of their rainfall and relative humidity (Martínez 1997, Martínez et al. 1998), these regions were declared to be at high risk of potential infection in the short term (Figure 1).

They are characterized by rainfall in excess of 1500 mm/yr, a relative humidity above 79% and a mean temperature between 25 and 28°C (Figures 1 and 3), which is significant in view of the relationship established between climate and the incidence and development of the disease (Fouré 1994, Gauhl 1994, Mobambo 1995). They are very different from the Maracay region where the mean rainfall is 922 mm/yr with a 6-month dry season. This situation has enabled the establishment of a model for comparing two totally different agro-ecological states with which are correlated critical levels of the severity reached by the disease. This model serves as a reference for introducing control measures on the basis of climatic conditions and to prevent possible spread into zones possessing similar characteristics (Martínez et al. 2000).

Fouré (1994) mentions relationships existing between climatic parameters and the spread of the disease which permit a better understanding of the dynamics of the epidemic in production zones and of its potential for initiating future infection. The liberation of ascospores is rapid during rain because of the presence of a film of residual water on the upper surface of the leaves, whose lower surface exhibits more lesions. Dead leaves which remain attached to the plant therefore represent an excellent source of inoculum (Gauhl 1994). As to the temperature, it is estimated that the ascospores of *Mycosphaerella fijiensis* germinate between 10 and 38°C, with an optimum at 27°C, and noting that the relative growth rate of the germination tubes (hyphae) falls rapidly at temperatures below 20°C (Pérez and Mauri, cited by Pérez 1996). Concerning the effect of wind, it has been shown that the concentration of conidia in plantations is higher in the lowest air layers than on the leaves whereas the concentration of ascospores in the air is the same: this confirms the importance of the ascospores in the life cycle of the disease (Stover 1984, Gauhl 1994).

It remains to emphasize the presence of topographic accidents in well-defined geographical areas which is apparently correlated with the variation in climatic factors mentioned above. These topographic accidents therefore also affect the development and severity of the disease. The first report of the disease
occurred in the Lake Maracaibo area, where the high relative humidity may be due to the proximity of the lake and to the topography of the landscape of the region. These conditions are similar to those around Lake Valencia (the point of entry of the disease into the state of Aragua) and in the sectors close to the Caroni river, Hato Gil (Bolivar state). In the same way, the presence of the Andes cordillera and the interior mountain chain, which constitute a natural barrier to the passage of the fungal spores to adjacent regions, ought to have prevented the spread of the disease into these zones. However this has happened, and its appearance in these regions can only be due to the transport of infected material.

Influence of the disease on plantation management
Farm surveys and visits to different areas of the country have shown us that the biggest losses have occurred in fields where there was no control of weeds, nematodes or insects. Hanging, dried-up leaves were not removed and no fertilizer was used. There were also problems of irrigation and drainage and an unsatisfactory spacing of plants in the field. The growers are not in the habit of removing side shoots, nor of using chemicals to control diseases. They lack technical support and resources to buy agrochemicals and equipment. Finally, there are no producers’ organizations. With a low yield which is all consumed by the family, the alternatives for the small producer are to sell his plantation, change the crop or simply to abandon the farm completely (Martínez et al. 2000).

Medium-sized producers tend to adjust the area of their plantation if production costs increase, allowing them to obtain yields which are dependent on the amount of investment. Large producers succeed in living with the disease, as can be seen to the south of Lake Maracaibo, where there are associations of producers and firms which improve the quality of the product in the plantations where it is destined for the international market. That which is rejected for export is sold on the national or local market where there is no quality control (Martínez et al. 2000).

Changes in crop management observed in the presence of black Sigatoka
The presence of black Sigatoka in the country has resulted in radical changes in the way bananas are grown. The traditional approach, which is to manage the plantations as perennial crops, tends to have been gradually replaced by semi-perennial, and in some cases annual management of the crop, with high planting densities possibly in association with other short-term crops, giving an increase both in yield and in the diversity of the products obtained. This has been introduced thanks to research work carried out by INIA, and also to the growing importance of the organization among the producers.

In the course of all the field experiments the accent has been placed on efficient application of basic cultural practices, such as removal of hanging dead leaves and the use of fertilizer, practices which are not being applied at present even though it has been demonstrated that they help to reduce the amount of inoculum of the pathogen in the plantation and that they render the plant less vulnerable to fungal attack (Gauhl 1994).

Quite clearly one should try to reduce the use of chemicals and aim for the best possible way of living with the pathogen. An alternative solution which may be adopted is the use of resistant clones which can either be grown in commercial plantations in rows between the clones traditionally grown in the country (so as to reduce the quantity of inoculum available), or else as an entirely separate plantation, as is seen in the Ocumare region of Costa, in Aragua state. There they have chosen to grow plantain FHIA-21, whose fruit has a softer texture than that of 'Hartón gigante' and can be used for making "tostones", or chips, of excellent quality, much appreciated by the consumers, which has facilitated its introduction to the market. Likewise it should be noted that there are other possibilities for production such as the use of the hybrids FHIA-01, FHIA-02 and FHIA-03 which yield well and have a very good response to the disease.

Conclusions
1. The speed of spread of the pathogen through the country has increased rapidly: its passage from the western zone to the central zone took five years while the spread from the central zone to the eastern zone and the south needed only one. It seems clear that this development has been encouraged by man. Causing an increase of 40-45% in the costs of production, the disease has particularly affected small producers and cast doubt over the survival of their holdings. The advance of the disease through the national territory continued into the states of Bolívar, Delta Amacuro and Amazonas between 1997 and 2000.
2. The case of the state of Amazonas, on the frontier with Brazil, is particular. The banana and the plantain, grown by the indigenous communities, are major elements of their diet. The ecosystem of the region is fragile and there is com-
plex genetic and biological diversity. For this reason it is undesirable to use chemical control products and preferable to recommend introducing resistant clones which do not need the application of fungicides, even if the cultivars are not fully acceptable to the native consumers.

3. Today it is evident that the presence of black Sigatoka in the country has brought about radical changes in the agronomic management of plantations. The effective use of basic cultural practices, within the framework of integrated control, makes it possible to live with the pathogen, as has been shown by numerous research studies carried out by INIA.

Acknowledgements
We thank particularly Mr Daniel Muñoz for his valuable collaboration in collecting information in the field, Mr Marcos Sanoja, a specialist at the Corporación Venezolana de Guayana (CVG), Fundacte-Guayana for logistic support, the staff of the climatology section of the Venezuelan Air Force (FAV) at Maracay, the Ministry of the Environment and Natural Renewable Resources (MARNR) and the Polar Foundation, through INIBAP, Montpellier, France. We thank particularly Mr Daniel Muñoz Pargas and Edwuard Manzanilla for his valuable collaboration in collecting information in the field, Mr Marcos Sanoja, a specialist at the Corporación Venezolana de Guayana (CVG), Fundacte-Guayana for logistic support, the staff of the climatology section of the Venezuelan Air Force (FAV) at Maracay, the Ministry of the Environment and Natural Renewable Resources (MARNR) and the Polar Foundation, through INIBAP, Montpellier, France.

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Frequency of Paracercospora fijiensis and Pseudocercospora musae in Dominico hartón plantain

C. Lorena Cardona-Sanchez and J. Castaño-Zapata

Plantain (Musa sp.) is a subsistence crop and in many areas is the staple food for the population especially in rural areas, with an estimated national per capita consumption of 68.5 kg/annum. Production is mostly with minimal cultivation and as a result yellow Sigatoka and black Sigatoka have increased in severity and dissemination (Merchán 1998), reducing production by 50% (Burt et al. 1997).

Nationally 384 957 ha are cultivated, 1000 m above seal level. According to some reports, black Sigatoka, caused by Mycosphaerella fijiensis Morelet, is
found attacking plantain Dominico hartón in the district of Victoria (Caldas) 100 m above sea level. and is more aggressive than yellow Sigatoka, caused by Mycosphaerella musicola Leach, a disease which was displaced in less than six months (Merchán 1992). A similar behaviour was observed in the district of Pueblo Rico (Risaralda) 1560 m above sea level (Merchán 1992). According to the latest reports, black Sigatoka can affect plantain from sea level up to an altitude of 1940 m (Belacázar et al. 1994).

The Sigatokas are difficult to differentiate from their external symptoms in the field, and hence it is not possible to establish clearly which of the two diseases is more frequent when they occur together (Aguirre et al. 1998b). Microscopically, M. fijiensis and M. musicola are distinguished mainly by the morphological differences of the anamorphs, in particular the conidiophores and conidia characteristics, especially by the presence of scars present on conidiophores and conidia of Paracercospora fijiensis but absent from Pseudocercospora musae (Aguirre et al. 1998b).

Management of these diseases may be with chemical products, a practice which is not really applicable to the traditional system of crop production. In order to solve this problem more economical alternatives have been sought, such as host resistance to both diseases which results in a reduced sporulation of the causal organisms.

The study was carried out with the objective of determining the frequency of P. fijiensis and P. musae spores in plantain Dominico hartón which is susceptible to black and yellow Sigatoka diseases.

**Materials and methods**

The investigation was carried out in the Tolima department, 7 km from the District of Fresno, on the road from Manizales (Caldas) to Mariquita (Tolima) in the village of La Ceiba, Campoalegre estate, located at an altitude of 1250 masl and a temperature range of 18-25°C, a relative humidity of 65-100% and a rainfall of 1800 mm/annum.

First, 1600 plants of the clone Dominico hartón, produced in vitro and multiplied in the tissue culture laboratory, Departamento de Fitotecnía de la Facultad de Ciencias Agropecuaria de la Universidad de Caldas, were transplanted then acclimatized at the Montelindo farm of the University.

Evaluations were carried out weekly on 53 clones selected at random. Records were taken starting 13 September 1998, the time of flower initiation, until harvest on 13 March 1999. Impressions were taken weekly from leaves attacked by the Sigatokas with the aim of quantifying spore populations of the anamorph state of M. fijiensis (Figure 1) and M. musicola (Figure 2).

Leaf impressions were made with syringes of solidified agar in the form of a dispenser, which was prepared from a 5 ml disposable syringe from which the far end was removed forming a cylinder 1.26 cm diameter. The dispenser was filled with crystal violet agar, prepared by mixing 1 g bacteriological agar with 15 ml of a 1% solution of crystal violet in distilled water and 100 ml water. The mixture was autoclaved at 121°C for 15 min., after which 1 mg benomyl and two sensitivity discs of streptomycin (10 µg) were added (Aguirre et al. 1998b).

Conidial populations were quantified weekly by making impressions of each material evaluated, the conidia being removed by pressing the agar surface against the lesion at leaf stage 4-5 of the youngest leaf spotted. The cylinder of crystal violet agar and conidia was placed on a slide and transferred to a tray lined with a paper towel moistened with sterile water. The trays were covered with plastic bags and transferred to a hermetic polystyrene container (icopor).

Both fungi were identified and the conidia/cm² counted by means of a compound microscope (Olympus) with a 40 x objective.

The variables analyzed were the numbers of conidia/cm² of P. fijiensis and P. musae, temperature (maxima, means and minima), relative humidity and rainfall.

Each variable was subjected to analysis of variance, descriptive procedures for maximum, minimum and mean values, regression, Pearson's correlation and Chi-square test using the SAS (Statistical

![Figure 1](image1). Conidia of Paracercospora fijiensis, anamorph of Mycosphaerella fijiensis.

![Figure 2](image2). Conidia of Pseudocercospora musae, anamorph of Mycosphaerella musicola.)
Analysis System) statistical programme (SAS Institute 1980). Conidial numbers were transformed with Lnx+1, which best fits the behaviour of the data, where x is the number of conidia/cm².

Results and discussion
Analysis of variance of P. fijiensis and P. musae counts suggested highly significant differences for clones and dates of evaluation. The interactions between the two factors were significant for P. fijiensis and highly significant for P. musae, indicating that a high or low inoculum production depends on the planting material and the effects of environmental conditions on the development of each material (Table 1).

The processes of infection and inoculum production were favoured by rainy periods, and as rainfall increased so did the numbers of conidia shown by P. fijiensis and P. musae with two periods of maximum conidial production at 334 and 424 days after planting (dap). These coincided with the maximum rainfall recorded during the study, with an accumulated rainfall of 211.8 mm and 296.2 mm respectively (Figure 3A), which was in agreement with the studies of Aguirre et al. (1998a). The authors observed that accumulated rainfall was inversely related to the incubation period and development of black and yellow Sigatokas, and directly related to sporulation. This suggests that as weekly accumulated volume of rainfall increases, the incubation period and development of both Sigatokas declines and results in an increased disease severity and hence greater inoculum production of the causal fungi. From 424 dap the relationship between conidial number and rainfall started to decline, being particularly evident at 473 dap when, although there was an increased rainfall (192.5 mm), conidial production was very low because foliage was severely necrotic, and no healthy tissue remained available for infection.

Temperature and relative humidity remained fairly constant with an average of 21.5°C (Figure 3B) and 81% (figure 3C), conditions that are optimum for conidial production. In agreement with Mouliom Pefoura and Mourichon (1990) and Tapia (1993), cited by Porras and Pérez (1997), temperatures higher than 20°C favour conidial development in P. fijiensis. According to Stover (1965), temperatures higher than 22°C favour conidial production in P. fijiensis, with a temperature of 26°C being optimum (Stover

<table>
<thead>
<tr>
<th>Table 1. ANOVA components for the number of conidia of Paracercospora fijiensis and Pseudocercospora musae.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANOVA</strong></td>
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<tr>
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</tr>
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<td>Error</td>
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<td>Interaction of clone-date</td>
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<td>Model</td>
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<tr>
<td>Error</td>
</tr>
<tr>
<td>Clones</td>
</tr>
<tr>
<td>Dates of evaluation</td>
</tr>
<tr>
<td>Interaction of clone-date</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*: Denotes significant differences, p = 5%

**: Denotes highly significant differences, p = 1%

Note: Data transformed as square root number of conidia.

Figure 3. Total conidia of P. fijiensis and P. musae in relation to time for 53 clones and their relationship to climatic conditions.
A. Rainfall, B. Temperature and C. Relative humidity.
1965). A relative humidity of about 100% favours production and viability of spores, particularly when a water film is present on the leaf surface (Jacome and Schuh 1992).

Conidial populations of \textit{P. fijiensis} were always greater than those of \textit{P. musae} in a ratio of 2.3:1 (Table 2) hence confirming that black Sigatoka tends to displace yellow Sigatoka because of its greater aggressiveness; this is in agreement with the studies in the same region by Aguirre et al. (1998a) who demonstrated that black Sigatoka was more aggressive, occurring at times of the year when yellow Sigatoka disappeared, tending to be displaced by black Sigatoka. In general there was a marked direct correlation between conidial numbers of \textit{P. fijiensis} and \textit{P. musae} and rainfall, which is also in agreement with the studies of Aguirre et al. (1998a) who observed that fluctuations in the numbers of conidia trapped each week is highly correlated with rainfall.

Table 2. Mean conidia/cm² with time for \textit{P. fijiensis} and \textit{P. musae} and their standard deviations, and their agreement with rainfall (September 1998 – March 1999).

<table>
<thead>
<tr>
<th>Days after planting (dap)</th>
<th>Correlation (r)</th>
<th>Conidia of \textit{P. fijiensis}</th>
<th>Standard deviation</th>
<th>Conidia of \textit{P. musae}</th>
<th>Standard deviation</th>
<th>Weekly rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>319</td>
<td>0.7782 **</td>
<td>45</td>
<td>65.96</td>
<td>37</td>
<td>58.55</td>
<td>81.60</td>
</tr>
<tr>
<td>326</td>
<td>0.7466 **</td>
<td>42</td>
<td>55.10</td>
<td>16</td>
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<td>34.40</td>
</tr>
<tr>
<td>334</td>
<td>0.8464 **</td>
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<td>12</td>
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<td>95.80</td>
</tr>
<tr>
<td>340</td>
<td>0.8115 **</td>
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<td>58.84</td>
<td>13</td>
<td>20.71</td>
<td>9.70</td>
</tr>
<tr>
<td>347</td>
<td>0.6622 **</td>
<td>23</td>
<td>29.70</td>
<td>12</td>
<td>27.17</td>
<td>21.00</td>
</tr>
<tr>
<td>361</td>
<td>0.8557 **</td>
<td>13</td>
<td>11.35</td>
<td>7</td>
<td>8.61</td>
<td>44.00</td>
</tr>
<tr>
<td>375</td>
<td>0.6407 **</td>
<td>31</td>
<td>37.79</td>
<td>9</td>
<td>14.34</td>
<td>51.80</td>
</tr>
<tr>
<td>389</td>
<td>0.7373 **</td>
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<td>22.61</td>
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<td>4.80</td>
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<td>41.92</td>
<td>8</td>
<td>14.96</td>
<td>173.70</td>
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<tr>
<td>411</td>
<td>0.6903 **</td>
<td>15</td>
<td>20.72</td>
<td>5</td>
<td>8.69</td>
<td>42.20</td>
</tr>
<tr>
<td>418</td>
<td>0.7697 **</td>
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<td>18.44</td>
<td>4</td>
<td>6.55</td>
<td>25.30</td>
</tr>
<tr>
<td>424</td>
<td>0.6966 **</td>
<td>14</td>
<td>19.26</td>
<td>5</td>
<td>11.66</td>
<td>55.00</td>
</tr>
<tr>
<td>438</td>
<td>0.8575 **</td>
<td>14</td>
<td>21.65</td>
<td>2</td>
<td>3.78</td>
<td>51.90</td>
</tr>
<tr>
<td>452</td>
<td>0.6361 **</td>
<td>10</td>
<td>13.14</td>
<td>2</td>
<td>4.78</td>
<td>57.60</td>
</tr>
<tr>
<td>466</td>
<td>0.6028 **</td>
<td>5</td>
<td>6.05</td>
<td>2</td>
<td>3.68</td>
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<td>473</td>
<td>0.8074 **</td>
<td>4</td>
<td>6.08</td>
<td>2</td>
<td>4.35</td>
<td>89.30</td>
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<td>493</td>
<td>0.5474 **</td>
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<td>3.62</td>
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<tr>
<td>Mean</td>
<td>0.7971 **</td>
<td>20</td>
<td>11.39</td>
<td>7</td>
<td>8.61</td>
<td>44.00</td>
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</tbody>
</table>

** Correlations highly significant between the number of conidia of \textit{P. fijiensis} and \textit{P. musae} with time in relation to rainfall.

Table 3. Comparison of the mean number of conidia/cm² of \textit{P. fijiensis} and \textit{P. musae} in clones evaluated in relation to rainfall.

<table>
<thead>
<tr>
<th>Days after planting (dap)</th>
<th>Mean number conidia of \textit{P. fijiensis}</th>
<th>Mean number conidia of \textit{P. musae}</th>
<th>Rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>319</td>
<td>45*</td>
<td>37</td>
<td>81.60</td>
</tr>
<tr>
<td>326</td>
<td>42</td>
<td>17</td>
<td>34.40</td>
</tr>
<tr>
<td>334</td>
<td>26</td>
<td>12</td>
<td>95.80</td>
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<td>340</td>
<td>32</td>
<td>13</td>
<td>9.70</td>
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<td>347</td>
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<td>361</td>
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<td>389</td>
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<td>3</td>
<td>109.10</td>
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<td>22</td>
<td>8</td>
<td>173.70</td>
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<td>418</td>
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<td>424</td>
<td>14</td>
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<td>55.00</td>
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<tr>
<td>438</td>
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<td>2</td>
<td>51.90</td>
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<tr>
<td>452</td>
<td>10</td>
<td>2</td>
<td>57.60</td>
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<tr>
<td>466</td>
<td>5</td>
<td>2</td>
<td>103.20</td>
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<tr>
<td>473</td>
<td>4</td>
<td>1</td>
<td>89.30</td>
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<tr>
<td>493</td>
<td>3</td>
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<tr>
<td>Total</td>
<td>281</td>
<td>139</td>
<td>1266.00</td>
</tr>
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</table>

*Correlation coefficient was very low (r = 0.40656) (Figure 5), suggesting that the number of conidia/cm² of \textit{P. musae} did not depend on the behaviour of \textit{P. fijiensis} and vice versa, and that conidial production in each of the clones depended on the susceptibility of the material and environmental conditions, particularly rainfall. In spite of the higher numbers of \textit{P. musae} conidia, the numbers not always increasing or decreasing, the conidia of \textit{P. musae} followed the same pattern, but there was no direct or strong relationship between inoculum production of the two pathogens.

In general, the clones evaluated produced higher total numbers of conidia of \textit{P. fijiensis}, confirming that black Sigatoka tends to displace yellow Sigatoka (Table 3).
References

Mycosphaerella fijiensis

Morelet y M. musicola 

Leach en siete genotipos de 

Musa 

spp. en un área 

límite de expansión de la Sigatoka negra en la 

zona cafetera colombiana. Pp. 192-220 

in 

Memorias del Seminario Internacional sobre Producción de Plátano. Universidad del Quindío - Comité 

de Cafeteros del Quindío - SENA - INABAP - CORPOICA.


Mycosphaerella musicola 


Reacción de variedades mejoradas de plátano al ataque de Sigatoka negra (Mycosphaerella fijiensis Morelet). Pp. 192-214 in Mejoramiento 


ICA-CORPOICA. Creced-Quindío, Armenia, Colombia.


tance wind dispersal of the fungal pathogens caus-

ing Sigatoka diseases in banana and plantain. 


Comportamiento agroeconómico de plántulas de 
plátano clon Dominico hartón 

Musa 

AAB 

Simmonds, manejados bajo condiciones de almá-

cigo. Pp. 41-54 

in 

Mejoramiento de la producción 

del cultivo del plátano. Segundo informe técnico 

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Porras A. & L. Pérez. 1997. The role of temperature in the growth of the germ tubes of ascospores of Mycosphaerella spp., responsible for leaf spot 


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Figure 4. Frequency of P. fijiensis and P. musae with time and the relationship with rainfall.

Figure 5. Relationship between the number of conidia of P. musae and P. fijiensis.
Effects of the natural fungicide F20 on black Sigatoka disease (Mycosphaerella fijiensis Morelet) on plantain (AAB) and banana (AAA)

R. Sánchez Rodríguez, J.A. Pino Algora, C. Vallin Plous, M.E. Pérez Rodríguez, Y. Iznaga Sosa and F. Malpartida Romero

The presence of black Sigatoka disease (Mycosphaerella fijiensis) in Cuba since 1990 has resulted in increased costs of production in plantations of plantain and banana because of the increased frequency of aerial and ground-based sprays to control the causal organism. Thus, there is an urgent need to find alternatives with nationally produced products in order to reduce the costs of disease control.

The indiscriminate use of chemical products has resulted in side effects including, inter alia, the appearance of fungicide resistance in the causal organism, the formation of strains more virulent than indigenous strains and environmental contamination (Rodríguez and Jiménez 1985, Fullerton and Olsen 1991, Moullim Pefoura 1999).

The use of natural products obtained from microorganisms presents considerable advantages in comparison with commercial products, since their production is much less damaging to the ecosystem and in situ biodegradability results in compounds that are not toxic to the indigenous microflora. The search for new and different products of natural origin that do not contaminate the environment, for the control of pests and diseases, is an important alternative for sustainable agriculture.

Product F20 comprises two antibiotics: the streptothricins B and F. These antibiotics are produced mainly by microorganisms of the genus Streptomyces. The structure has an aminosugar (glucosamine) joined to a β-lysine peptide chain. Streptothricins F to A differ in the numbers of β-lysine residues in the peptide chain, from 1-β-lysine in streptothricin A to 10, from about DS = 3000 to about DS = 50 in clone 'CEMSA 3/4'; the rate of disease development declined, whereas for control plants, DS values varied between 1500 and 2500.

Graphs A and B show the similar behaviour of F20 and Tilt with DS values not significantly different (P>0.05), and a significant difference (P<0.01) of both applications in comparison with the control treatment. The effects of disease control of the products were also evident in the graphs as a reduction in the numbers of oscillations in DS values and by the amplitude of their fluctuations in both clones. For example, when treated in week 5, DS values declined steadily from weeks 5 and 10, from about DS = 3000 to about DS = 50 in clone 'CEMSA 3/4'; the rate of disease development declined, whereas for control plants, DS values varied between 1500 and 2500.

Analysis of the variable YLSS (Figure 2) showed no significant differences (P>0.05) between treatments with F20 or Tilt. However, with clone 'Parecido al Rey' and F20, minor disease symptoms, stage 1, were evident on leaf 9 (Pérez 1996).

Figure 3 showed that YLS could reach a value equal or greater than 9 before...
flower initiation, thus confirming that there were no effects on weight or premature fruit maturity in either clone; in Cuba a strong negative correlation between the leaf area affected and the YLS has been observed (Pérez et al. 1993, Pérez 1996).

All the data analyzed above suggest the use of F20, produced from natural origins and mixed with mineral oil and commercial detergent as an emulsion, for the control of black Sigatoka disease in banana and plantain crops. F20 is superior to synthetic chemicals in terms of environmental effects. Attention is
drawn to the difference in behaviour between the two clones, with 'CEMSA 3/4' having the higher infection. However, in order to avoid the possibility of fungal resistance, it is important that this product should form part of an integrated programme in combination with other antifungal products (Pérez 1996, Romero 1997).

Conclusions

• Product F20 showed no significant differences in comparison with the commercial product Tilt in its effectiveness to control black Sigatoka.

• Maximum effectiveness of F20 was obtained with an emulsion of mineral oil and commercial detergent, and the effect was maintained for 3 or 4 weeks from the time of application.

• Application at doses of 80-200 g streptomycin/ha controlled black Sigatoka in field plantations, irrespective of time of year. There are advantages in comparison with synthetic chemical products because its biodegradability in situ results in compounds that are not toxic to the indigenous microflora and it has a lower toxicity.

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Seasonal fluctuations of Radopholus similis and Pratylenchus coffeae in certain cultivars of banana

P. Sundararaju

The lesion-producing nematodes such as Radopholus similis and Pratylenchus coffeae are considered to be the economically important nematode pests of banana and are widely distributed in South India (Koshy et al. 1978, Rajendran et al. 1979). The burrowing nematode, Radopholus similis enjoys wide geographical distribution in the tropical and subtropical banana-growing regions of the world. In India, the first occurrence of the nematode was reported on banana from Palghat District of Kerala (Nair et al. 1978), subsequently this nematode was reported from banana in South India (Koshy et al. 1978), Gujarat (Sethi et al. 1981), Maharashtra (Darekar et al. 1981), Madhya Pradesh (Tiwari et al. 2000), Goa (Koshy and Sosamma 1988), Lakshadweep Islands (Sundararaju 1990), Manipur (Anandi and Dhanchand 1992), Orissa (Mohanty et al. 1992), Tripura (Mukherjee et al. 1994) and Bihar, Uttar Pradesh and Nagaland (Khan 1999).

The root-lesion nematode, Pratylenchus coffeae is reported to have spread to different banana-growing regions through the infested corm. In India, the nematode is known to occur on plantain (AAB) in South India, Gujarat, Orissa, Bihar and Assam (Sundararaju 1996). P. thornei, the other important species was found to infest banana plants from Assam only (Choudhury and Phukan 1990). Crop losses caused by nematodes to bananas are very high, with average annual yield losses estimated at about 20% worldwide (Sasser and Freckman 1987). Soil temperature at a depth of 30 cm did not influence population size (Jiménez 1972). The populations were found to fluctuate between samples, trees, months and years. However, there were definite periods for occurrence of maximum and minimum populations within a year. An extensive survey carried out by Sundararaju (1996) from different banana-growing regions in the country indicated the presence of 17 genera of plant parasitic nematodes. Among them, the lesion nematodes, Radopholus similis and Pratylenchus coffeae are the predominant species found to occur in different cultivars of banana in various intensities. The burrowing nematode-infested field exhibited severe root rotting, resulting in serious economic losses. Yield was reduced up to 25-35% in the burrowing nematode-infested field compared to nematode-free plantations. Crop losses due to the root-lesion nematode, Pratylenchus coffeae in banana cv. Nendran were reported to be 25.4% (Sundararaju et al. 1999). Therefore, studies were initiated to determine the seasonal fluctuations of these nematode species in southern India.
populations in different cultivars of banana roots by periodic sampling of nematode-infested banana plants at the National Research Centre for Banana (NRCB) Farm. The main objective of the study was to find out the activity peaks, in terms of highest and lowest populations of these nematode parasites, in the rhizosphere so that while devising the management schedules, the findings of the work could be considered.

Materials and methods
In order to study the population fluctuation of lesion nematodes, the three banana cultivars Kalyan bale (AB), Alukkal (ABB) and Kalibow (AAB), which are highly susceptible to *R. similis*, and a variety Nendran (ABB), which is highly susceptible to *P. coffeae*, were selected at NRCB Farm, Podhavur, Trichy, Tamil Nadu. The nematode-infested field was selected for this study and the tested banana cultivars were grown in the field and alluvial soil condition. Samples of both soil (250 cc) and root (10 g) were collected from the base of the mother plants at monthly interval from the 5th month up to harvesting stage during 1997-98. Tender, main feeder roots of white to creamy-white colour, with reddish-brown cortical lesions, were collected from the base of the plants. Care was also taken to collect only the above-mentioned type of roots known to harbour the maximum number of lesion nematodes. Root samples washed thoroughly and cut into 2-2.5 cm pieces and later sliced into 8 longitudinal pieces were left in 15 cm-Petri dishes containing 150 ml of tap water for 72 hrs at 10-14°C in a refrigerator for nematode extraction (Koshy et al. 1975). Soil samples were processed as per Cobb’s sieving method followed by the modified Baermann’s funnel method for estimation of nematode populations. Soil temperature at 15 cm depth was recorded from the fields daily at 7 am. The mean temperature, soil moisture and data on cumulative rainfall were correlated with the nematode population density in the sample.

Results and discussion
It is seen from Figure 1 that a drastic increase of *R. similis* population was noticed in all three cultivars during the months of November to April; it later decreased to a negligible level from May to October which was in agreement with Shaﬁce and Mendez (1975). It is interesting to observe that the maximum nematode population was recorded in April from all the cultivars: Kalyan bale (86/g root), Alukkal (78/g root) and Kalibow (68/g root), and the minimum in July in cv. Kalyan bale (20/g root). Analysis of the soil samples also revealed the same trend as in the case of root samples with maximum population occurring during the month of November to April with maximum rainfall and soil moisture during the period. In the case of *P. coffeae* on variety Nendran the maximum population was recorded from

![Figure 1. Population fluctuation of Radopholus similis in roots of banana.](image1)

![Figure 2. Population fluctuation of Pratylenchus coffeae in roots of banana (cv. Nendran).](image2)

![Figure 3. Total monthly rainfall, mean temperature, soil moisture and relative humidity at NRCB Farm, Podhavur, during the experimental period.](image3)
and gradually decreased from January to June (Figure 2). This clearly shows that the population build-up of R. similis and P. coffeae would greatly vary depending upon the season and other ecological conditions such as rainfall, soil temperature, soil moisture and availability of susceptible roots which play their own roles in the population build-up.

Acknowledgement
The author is thankful to Dr H.P. Singh, former Director, NRCB, Trichy for providing necessary facilities. Technical assistance given by Mr T. Sekar is duly acknowledged. This research work was carried out within the framework of the NRCB research programmes.

References
Vilardebo A. 1976. Nematodes of Pratylenchus spp. correlated with rainfall (Cooke and Draycott 1971). The behaviour of P. coffeae in relation to soil temperature and rainfall was similar to that of P. crenatus and P. penetrans in corn (Miller et al. 1972). Lack of moisture coupled with high summer temperature during April to August was found to be unfavourable for the prevalence of P. coffeae in oil palms (Sundararaju and Ratnakaran, in press). The present investigation is in agreement with Kumar (1984) who reported that higher population of P. coffeae was recorded during the month of October to December which is the period of high rainfall and increased root activity in coffee plants.

Similar observations were reported in the burrowing nematode R. similis on citrus (DuCharme and Suit 1967), banana (Vilardebo 1976), coconut and arecanut (Koshy and Somasama 1978).

Figure 1 indicates that the R. similis population fluctuates between the months. A steady increase of R. similis population was recorded during the months of November-January and gradual decrease was recorded in February and March, whereas a drastic increase of nematode population was recorded in April in cv. Kalyan bale (Figure 1). A similar trend was noticed in cvs. Alukkal and Kalibow (Figure 1).

In the case of P. coffeae a steady increase of nematode population was recorded from September to December and October to December and the minimum population from May to August (Figure 2). Regarding the average population for a month, it is seen that a maximum of 92 per gram root was recorded in December, whereas it was only 23 per gram in January. The root-lesion nematode population from soil samples also showed the same trend as in the case of root samples with maximum population during the months October-December and minimum population during the months May-August. Peak rainfall occurred during the Northeast monsoon (September to December) with an average rainfall of 140 mm. The soil temperature at 15 cm depth recorded from fields varied from 18-37.5°C. Analysis of the moisture content revealed that it was maximal in those months where maximum nematode populations were recorded (Figure 3). Rainfall also influences the growth of roots. Thus, with the increase in the availability of root system, there was an increase in the activity of R. similis during November to April and of P. coffeae during October to December.

Fluctuations in the populations of Pratylenchus spp. were correlated with rainfall (Cooke and Draycott 1971). The behaviour of P. coffeae in relation to soil temperature and rainfall was similar to that of P. crenatus and P. penetrans in corn (Miller et al. 1972). Lack of moisture coupled with high summer temperature during April to August was found to be unfavourable for the prevalence of P. coffeae in oil palm (Sundararaju and Ratnakaran, in press). The present investigation is in agreement with Kumar (1984) who reported that higher population of P. coffeae was recorded during the month of October to December which is the period of high rainfall and increased root activity in coffee plants.

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Host plant response of Pisang Jari Buaya and Mysore bananas to Radopholus similis

Plant parasitic nematodes are a major constraint of banana production worldwide (Gowen and Quénéhervé 1990). Nematode infection can interfere with nutrient and water uptake and transportation, resulting in slow growth, reduced fruit filling and uptake and transportation, resulting can interfere with nutrient and water uptake and transportation, resulting in slow growth, reduced fruit filling and uptake and transportation, resulting can interfere with nutrient and water uptake and transportation, resulting can interfere with nutrient and water uptake and transportation, resulting can interfere with nutrient and water uptake and transportation, resulting can interfere with nutrient and water uptake and transportation, resulting can interfere with nutrient and water uptake and transportation. Among the nematodes attacking banana, Radopholus similis (Cobb Thorne) is considered the most destructive species (Sarah et al. 1996).

The possibilities of controlling nematodes in bananas are limited because bananas are usually grown as a permanent crop by small-scale farmers and sources of resistance have proved hard to find. Resistance to R. similis has been reported in 'Pisang Jari Buaya' (Musa AA—Pisang Jari Buaya group) and 'Yangambi Km5' (Musa AAA—Ibota group) (Pinochet 1988, Viaene et al. 1998, Fogain and Gowen 1998, Stoffelen 2000). The clone 'SH-3142' derived from a genotype belonging to the Pisang Jari Buaya group and 'SH-1734' was found to be highly resistant to R. similis (Pinochet and Rowe 1979, Pinochet 1988). Moreover, some 'Pisang Jari Buaya' expressed favourable agronomic features similar to those of commercial banana.

The Mysore banana (Musa AAB) is a very popular and delicious dessert. Information on resistance and/or tolerance to R. similis of Mysore bananas is scarce. When testing 17 AAB Musa genotypes, Fogain (1996) reported that none of the plants were immune, including 'Pisang Ceylan', the only cultivar belonging to the Mysore group. The objective of our study was to further investigate the host plant response of Musa genotypes from the Pisang Jari Buaya and Mysore groups to R. similis population from Costa Rica, to find additional sources of resistance to the burrowing nematode.

Throughout the study, the terminology of Bos and Parlevliet (1995) concerning resistance and susceptibility of host plants to pathogens and the methodology for nematode resistance screening in Musa as described by Speijer and De Waele (1997) were used.

**Materials and methods**

**Preparation of banana plants**

Thirteen diploid (AA) banana genotypes belonging to the Pisang Jari Buaya group (Experiments 1 and 2, see Tables 1 and Table 2) and five triploid (AAB) banana genotypes from the Mysore group (Experiment 3, see Table 3) were included in the study. Two triploid (Musa AAA) bananas, 'Grande Naine' and 'Yangambi Km5', were included as reference genotypes because of their high susceptibility and resistance to R. similis, respectively. The Musa genotypes used in the experiments were provided by the INIBAP Transit Centre (ITC) at the Catholic University of Leuven. After proliferation, regeneration and rooting (Banerjee and De Langhe 1985), each in vitro propagated banana plantlet with 3-4 leaves and 5-6 roots was transplanted in a 1-litre (12 cm-diameter) plastic pot containing about 1000 cm² autoclaved substrate of peat and quartz (2:1). To keep a high humidity, the pots were placed under a plastic cover, which was slightly opened after 2 weeks and removed after 4 weeks. The greenhouse conditions were maintained at 25-30°C and 70-80% relative humidity with a 12-hour photoperiod. The pots were irrigated as needed and fertilized with a hydroponics solution (Swennen et al. 1986) every 3 weeks after nematode inoculation. The plants were inoculated with nematodes either 4 weeks after planting for the Pisang Jari Buaya group, or 8 weeks after planting for the Mysore group, since the number of nematodes was too low in the experiment with Mysore genotypes.

**Preparation of nematode inoculum**

The R. similis population used in the experiments was obtained from infected banana roots of 'Valery' (Musa AAA) at Talamanca in Costa Rica. The population was reared monoxenically on carrot discs and incubated at 28°C in the dark for several generations (Moody et al. 1973, Pinochet et al. 1995). The carrot discs were blended twice for 10 s (with 5 s interval) and poured through 106 and 25 µm pore sieves. Carrot tissue collected on the 106 µm pore sieve was discarded, while the nematodes were collected from the 25 µm pore sieve. A suspension of 1000 living vermiform nematodes was poured in three holes made in the substrate around the base of each plant. After inoculation, the holes were covered.

**Host plant response observations**

Eight weeks after inoculation, the plants were harvested to observe the response of the different banana genotypes to R. similis. The following data were recorded:

**Root necrosis percentage**

The procedure followed was that described by Speijer and De Waele (1997). Five 10 cm-pieces of functional primary roots were collected and sliced longitudinally. The percentage of root necrosis per root half was 20%, giving a maximum root necrosis of 100% for the five root-halves together.

**Nematode population densities**

The entire root system, including the 5 roots segments observed for necrosis, was weighed and cut into 2 cm-pieces. Fifteen grams of fresh roots were taken randomly and macerated three times for 10 s with 5 s intervals. The mixture was poured through a series of 250-106-40 µm pore sieves and the sieves were rinsed with tap water. Nematodes remaining on the 40 µm pore sieve were collected in a beaker with distilled water. Nematodes were counted in 6 ml aliquots of each sample using a binocular microscope.

**Experimental design and data analysis**

Three experiments were conducted, based on a completely randomized design, with either eight replicates for each genotype (Pisang Jari Buaya group, Experiment 1, Table 1; Mysore group, Experiment 3, Table 3) or nine replicates (Pisang Jari Buaya group, Experiment 2, Table 2). Prior to statistical analysis, the percentage of root necrosis was transformed to arcsin
Table 1. Reproduction of Radopholus similis (Costa Rica population) on 8 diploid (Musa AA) banana genotypes belonging to the Pisang Jari Buaya group and on the reference genotype ‘Grande Naine’ measured 8 weeks after inoculation with 1000 vermiform nematodes per plant.

<table>
<thead>
<tr>
<th>Musa genotype</th>
<th>Genome</th>
<th>ITC number</th>
<th>Fresh root weight (g)</th>
<th>Root necrosis (%)</th>
<th>Nematodes per 1 g fresh roots</th>
<th>Nematodes per root system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huwundu</td>
<td>AA</td>
<td>0308</td>
<td>35.3</td>
<td>22.4</td>
<td>1050 a</td>
<td>36188 a</td>
</tr>
<tr>
<td>Morong Datu</td>
<td>AA</td>
<td>0309</td>
<td>41.0</td>
<td>14.5</td>
<td>851 a</td>
<td>33526 a</td>
</tr>
<tr>
<td>Morong Princessa</td>
<td>AA</td>
<td>0310</td>
<td>29.9</td>
<td>30.5</td>
<td>b 972 a</td>
<td>26022 a</td>
</tr>
<tr>
<td>Pisang Rotan</td>
<td>AA</td>
<td>0313</td>
<td>41.1</td>
<td>16.6</td>
<td>a 794 a</td>
<td>29033 a</td>
</tr>
<tr>
<td>Pisang Tunjuki</td>
<td>AA</td>
<td>0315</td>
<td>44.2</td>
<td>7.9</td>
<td>a 255 a</td>
<td>10770 a</td>
</tr>
<tr>
<td>Saing Todloh</td>
<td>AA</td>
<td>0316</td>
<td>36.0</td>
<td>6.8</td>
<td>a 297 a</td>
<td>10038 a</td>
</tr>
<tr>
<td>Unnamed</td>
<td>AA</td>
<td>0318</td>
<td>43.2</td>
<td>11.3</td>
<td>a 442 a</td>
<td>17151 a</td>
</tr>
<tr>
<td>Umbarm</td>
<td>AA</td>
<td>0317</td>
<td>32.6</td>
<td>17.1</td>
<td>a 495 a</td>
<td>16030 a</td>
</tr>
<tr>
<td>Grande Naine</td>
<td>AAA</td>
<td>1256</td>
<td>52.0</td>
<td>9.3</td>
<td>a 528 a</td>
<td>23052 a</td>
</tr>
</tbody>
</table>

ITC = INIBAP Transit Centre.

Original data are presented, but data of nematode numbers were transformed to log10 (x+1). All data percentage were converted to arcsin (x/100) for statistical analysis. Means in the same column followed by the same letter are not significantly different (P ≤ 0.05) according to the Tukey HSD test.

Table 2. Reproduction of Radopholus similis (Costa Rica population) on 5 Pisang Jari Buaya genotypes, Yangambi Km5 and on the reference genotype ‘Grande Naine’ measured 8 weeks after inoculation with 1000 vermiform nematodes per plant.

<table>
<thead>
<tr>
<th>Musa genotype</th>
<th>Genome</th>
<th>ITC number</th>
<th>Fresh root weight (g)</th>
<th>Root necrosis (%)</th>
<th>Nematodes per 1 g fresh roots</th>
<th>Nematodes per root system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabah Gabah</td>
<td>AA</td>
<td>0307</td>
<td>74.4</td>
<td>11 a</td>
<td>588 c</td>
<td>44571 b</td>
</tr>
<tr>
<td>Pisang Gigi Buaya</td>
<td>AA</td>
<td>0310</td>
<td>64.0</td>
<td>8.4</td>
<td>a 741 c</td>
<td>45914 b</td>
</tr>
<tr>
<td>Pisang Jari Buaya</td>
<td>AA</td>
<td>0312</td>
<td>54.9</td>
<td>11.9</td>
<td>a 1053 c</td>
<td>56541 b</td>
</tr>
<tr>
<td>SH-3142</td>
<td>AA</td>
<td>0425</td>
<td>52.7</td>
<td>8.9</td>
<td>a 108 a</td>
<td>5941 a</td>
</tr>
<tr>
<td>Pisang Sipulu</td>
<td>AA</td>
<td>1308</td>
<td>60.6</td>
<td>8.9</td>
<td>a 579 bc</td>
<td>34355 b</td>
</tr>
<tr>
<td>Yangambi Km5</td>
<td>AAA</td>
<td>1123</td>
<td>57.2</td>
<td>7.8</td>
<td>a 120 ab</td>
<td>6384 a</td>
</tr>
<tr>
<td>Grande Naine</td>
<td>AAA</td>
<td>1256</td>
<td>43.9</td>
<td>25 b</td>
<td>2041 c</td>
<td>87763 b</td>
</tr>
</tbody>
</table>

ITC = INIBAP Transit Centre.

See note in Table 1.

Table 3. Reproduction of Radopholus similis (Costa Rica population) on 5 triploid (Musa AAB) banana genotypes belonging to the Mysore group and on the reference genotype ‘Grande Naine’ measured 8 weeks after inoculation with 1000 vermiform nematodes per plant.

<table>
<thead>
<tr>
<th>Musa genotype</th>
<th>Genome</th>
<th>ITC number</th>
<th>Fresh root weight (g)</th>
<th>Root necrosis (%)</th>
<th>Nematodes per 1 g fresh roots</th>
<th>Nematodes per root system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thap M aeo</td>
<td>AAB</td>
<td>1301</td>
<td>101.3</td>
<td>17.3</td>
<td>a 852 ab</td>
<td>82849 abc</td>
</tr>
<tr>
<td>Gorolo</td>
<td>AAB</td>
<td>0723</td>
<td>45.6</td>
<td>22.8</td>
<td>a 579 ab</td>
<td>32562 a</td>
</tr>
<tr>
<td>Pisang Ceylan</td>
<td>AAB</td>
<td>0650</td>
<td>58.3</td>
<td>29.6</td>
<td>abc 804 ab</td>
<td>46827 abc</td>
</tr>
<tr>
<td>Lady Finger (South Johnstone)</td>
<td>AAB</td>
<td>0583</td>
<td>51.8</td>
<td>36.9</td>
<td>c 679 a</td>
<td>38616 ab</td>
</tr>
<tr>
<td>Lady Finger (Nelson)</td>
<td>AAB</td>
<td>0582</td>
<td>87.8</td>
<td>33.1</td>
<td>bc 1128 ab</td>
<td>99009 bc</td>
</tr>
<tr>
<td>Grande Naine</td>
<td>AAA</td>
<td>1256</td>
<td>83.9</td>
<td>42.5</td>
<td>c 1552 b</td>
<td>127439 c</td>
</tr>
</tbody>
</table>

ITC = INIBAP Transit Centre.

See note in Table 1.

Original data are presented, but data of nematode numbers were converted to log10 (x+1). All data were subjected to analysis of variance (ANOVA) and means of the parameters were compared using the Tukey HSD test at P ≤ 0.05.

Results and discussions

The results obtained from the Pisang Jari Buaya group are presented in Tables 1 and 2. In Table 1, no significant differences were observed in nematode numbers per root system or per 1 g fresh roots and root necrosis percentage between the Pisang Jari Buaya genotypes and ‘Grande Naine’. Among the Pisang Jari Buaya genotypes, root necrosis percentage was significantly higher in ‘Morong Princessa’ compared with ‘Pisang Tunjuki’ and ‘Saing Todloh’. In general, reproduction of ‘Pisang Ceylan’, ‘Lady Finger’ (South Johnstone) and Lady Finger (Nelson) did not differ significantly from those in ‘Grande Naine’.

According to Price (1994) and Price and McLaren (1995), AAB Musa genotypes are susceptible to R. similis when examined in field trials. Unfortunately, genotypes of the Mysore group were not included in their trials. Our study confirms previous reports (Stanton 1994, Fogain et al. 1996) that ‘Lady Finger’...
(Nelson), 'Lady Finger' (South Johnstone) and 'Pisang Ceylan' are susceptible to *R. similis*.

**Acknowledgements**

The authors would like to thank the INIBAP Transit Centre (ITC) at the Catholic University of Leuven for supplying the Musa genotypes and the equipment for completing this research. The Flemish Interuniversity Council (VL.I.R.) is gratefully acknowledged for funding scholarships for Ms Duong Thi Minh Nguyen and Ms Nguyen Thi Tuyet to complete this study as part of their MSc thesis in the Postgraduate International Nematology Course.

**References**


**Effect of three arbuscular mycorrhizal fungi on root-knot nematode (Meloidogyne spp.) infection of Musa**

A. Elsen, S. Dedeker and D. De Waele

Arbuscular mycorrhizal (AM) fungi are obligate symbionts of plants that biotrophically colonize the root cortex and develop an extramatrical mycelium which helps the plant acquire water and mineral nutrients from the soil. AM fungi also may protect plants against soil-borne plant pathogens, including nematodes. Several studies have addressed the associations between AM fungi and root-knot nematodes, which are considered the most important nematodes in the western hemisphere on temperate agricultural crops. Many mycorrhizal associations are reported to have a suppressive effect over sedentary endoparasitic nematodes. In some crops this effect is significant enough to consider mycorrhizal infection as a more or less effective means of biological control (Pinchot et al. 1996).
In bananas, only a few studies were carried out on the effects of AM fungi on nematode development. Radopholus similis populations in the roots as well as in the soil were suppressed in mycorrhizal plants compared to non-mycorrhizal plants (Umesh et al. 1988). Under in vitro conditions, using Ri T-DNA transformed Daucus carota roots, a R. similis population was suppressed with 50% in the presence of AMF (Elsen et al. 2001). Pinochet et al. (1997) reported that mycorrhizal colonization did not effect nematode build-up in the roots, although plants infected with both Melioideyne javanica and Glomus intraradices were more galled.

In this experiment, three Glomus species (G. mosseae, G. macrocarpum and G. caledonium) were tested on the Musa cultivar Williams (ITC0570) for their effect on Melioideyne javanica, a root-knot nematode population isolated from banana in Morocco. Tissue-culture derived plantlets were acclimatized in 1-litre pots filled with sterilized soil in the greenhouse. During transplant the plantlets of the mycorrhizal treatment were mycorrhized with soil inoculum, consisting of ± 1850 spores and 0.25 g mycorrhized roots from Allium porrum. After one month, the plants were inoculated with a mixture of 5000 M. javanica juveniles and eggs. The experiment was planned as a 4 x 2 randomly factorial design with 8 replicates per treatment: AM fungi (- AM, G. mosseae, G. macrocarpum and G. caledonium) x M. javanica (+ M. javanica, - M javanica). Three months after planting, the ‘Williams’ plants were harvested and assessed for mycorrhizal colonization and nematode damage/development. A sub-sample of the roots was stained with 0.05% trypa blue in lactic acid (Koske and Gemma 1989), in order to determine the mycorrhizal colonization. The galls on the roots were counted in a 5 g sub-sample after staining with phloxine B (Hadisoeganda and Sasser 1982).

Effect of the AM fungi on plant growth

The AM fungi had no effect on the plant growth since shoot weight, shoot diameter, plant height and root weight did not differ among the treatments (data not shown). In general, mycorrhization of banana plants resulted in a better plant growth compared to non-mycorrhizal plants (Declerck et al. 1994, 1995). Although, in some cases, it has been observed that the establishment of the symbiosis resulted in a negative or neutral effect on plant growth as long as the mycorrhizal colonization was not well developed (Jakobsen 1998). Therefore at the time of harvest, the root colonization by the three Glomus strains tested was relatively low. This may partly explain why in this experiment no effect on plant growth was observed. In addition, it is important to note the differences in colonization among the Glomus species in the plants without nematodes. Higher colonization was observed with G. mosseae as compared to G. caledonium and G. macrocarpum. Such differences were also reported in literature (Declerck et al. 1994, 1995). Glomus mosseae was shown the more infective on ‘Williams’ and other cultivars, as compared to G. macrocarpum (Declerck et al. 1995).

Effect of AM fungi on nematode reproduction

Glomus caledonium and G. macrocarpum significantly reduced galling in the roots, while for G. mosseae this reducing effect was not significant (Table 1). In literature, results are contradictory: according to Pinochet et al. (1997), Glomus intraradices did not reduce nematode build-up of M. javanica and resulted in more galled roots compared to non-mycorrhized roots. In contrast, G. mosseae suppressed root galling and nematode build up of Melioideyne incognita (Jaizme-Vega et al. 1997).

Effect of M. javanica on mycorrhizal development

Melioideyne javanica significantly decreased the intraradical development of G. mosseae. For G. macrocarpum and G. caledonium no such effect was observed: the presence or absence of the root-knot nematode had no effect on internal root colonization. In similar experiments, root-knot nematodes had no effect on the percentage root colonization in mycorrhizal plants (Pinochet et al. 1997, Jaizme-Vega et al. 1997).

Table 1. Mycorrhization and effect of mycorrhization on reaction of “Williams” roots to infection with Melioideyne javanica.

<table>
<thead>
<tr>
<th>% mycorrhized root tissue</th>
<th>Galls / 5 g roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>- AM – M. javanica</td>
<td>/</td>
</tr>
<tr>
<td>- AM + M. javanica</td>
<td>/ 41 ± 12 b</td>
</tr>
<tr>
<td>G. mosseae – M. javanica</td>
<td>29 ± 10 b</td>
</tr>
<tr>
<td>G. mosseae + M. javanica</td>
<td>23 ± 12 a</td>
</tr>
<tr>
<td>G. macrocarpum – M. javanica</td>
<td>14 ± 3 a</td>
</tr>
<tr>
<td>G. macrocarpum + M. javanica</td>
<td>15 ± 5 a</td>
</tr>
<tr>
<td>G. caledonium – M. javanica</td>
<td>22 ± 5 a</td>
</tr>
<tr>
<td>G. caledonium + M. javanica</td>
<td>16 ± 6 a</td>
</tr>
</tbody>
</table>

Data are means of 8 replications. Means in the same columns followed by the same letter do not differ according to Tukey’s multiple range test (P = 0.05).

Conclusion

The results of this experiment suggest a suppressive effect of the three Glomus strains studied over the rook-knot nematode M. javanica. Mechanisms involved in nematode suppression are still a matter of speculation. However some major factors are likely to be involved: enhanced nutrient status of the plant, biochemical changes in plant tissue (increase in chitinase, amino acids, peroxidase and phytoalexins), anatomical changes (increased lignification), stress alleviation, microbial changes in the rhizosphere and induced changes to root morphology (increased branching, larger proportion of higher order roots) (Hooker et al. 1994). Further study is needed to confirm the suppressive effect of the AM fungi over the root-knot nematodes and to reveal the mechanisms involved.

References

In Cuba a total of 108 700 ha are cultivated with Musaceae, 32 800 ha with cultivars of banana subgroup Cavendish (AAA), 13 800 ha with plantain (AAB) and 62 000 ha with varieties of type Burro/Bluggoe (ABB). Of the 32 800 ha of banana plantations, 13 800 ha are cultivated with localized microjet irrigation systems, and they must therefore remain in place for the next five years. However there is interest in replanting these areas with tetraploid hybrids developed by the Fondación Hondureña de Investigación Agrícola (FHIA), and which are resistant to pests and diseases.

The nematode species most commonly found in our plantations are Radopholus similis, Pratylenchus coffeae, Helycotylenchus multicinctus, Meloidogyne spp. and Rotylenchulus reniformis, the first three being the most important in Cuba (Pérez et al. 1984). The pathogenicity of nematodes has usually been established as the population density found in the roots. However, evidence on the relationship of population density of nematodes to damage in the crop is contradictory. Over the last years plant lodging and root necrosis in Cuba have been recorded where low populations of nematodes were very low.

Interactions at the root level between R. similis and species of fungi belonging to the genera Cylindrocladium and Acremonium, which contribute to or increase damage by the nematode, are well documented (Booth and Stover 1981, Loridat 1989, Sarah 1990). Such associations have been found in the majority of soils infested with nematodes in some of the Antilles islands. In Cuba there have been no studies to investigate and quantify such relationships at the level of the root. However the relationship between nematode populations, root damage and plant development is not strong.

The purpose of the present study was to identify the species of fungi associated with root necrosis of different clones of banana and plantain in plantations in Cuba.

Material and methods

Samples were taken from banana plantations located in the provinces of Pinar del Río, La Habana, Matanzas, Villa Clara, Ciego de Ávila, Camagüey, Cienfuegos, Santiago de Cuba and Guantánamo.

Samples were taken from necrotic roots of plants of Gran enano (AAA), Gros Michel (AAA), CEMSA 3/4 (AAB) and Burro CEMSA/ Bluggoe (ABB), some of them associated with plants that had fallen over (lodged) apparently as a result of nematode attack. From each field 10 plants were selected at random, holes 20 x 20 x 20 cm excavated 10 cm from the pseudostem and five affected roots removed.

The roots were washed and necrotic fragments, typical of R. similis attack, disinfected in 1% hypochlorite for two minutes and cultured on water-agar supplemented with 50 μg/ml streptomycin. Blocks of agar and fungal growth were transferred to tubes of PDA, and incubated when the fungal species were ready for identification. Fusarium species were identified according to the key of Booth (1981). Cylindrocarpon species were identified according to the keys of the CMI edited by CAB.

The relative frequency of each species present at each site was determined in relation to the total numbers of isolates obtained from the different sites.

Results and discussion

A total of 59 isolates of endophytic fungi were obtained from the tissue of roots apparently necrotic as a result of R. similis. The species are described in Table 1.

Table 1

| Species of Cylindrocarpon musae and Fusarium oxysporum Schlect. were isolated from almost all samples from all the sites. F. oxysporum was the most frequently isolated species (45.6% total isolates), followed by C. musae (19.2% total isolates). F. equiseti (Corda) Sacc. was also isolated but with less frequency. The results were similar to those of Pocasangre (2000) who found that Fusarium species were predominant in soils from Cuba, Costa Rica, Guatemala and Honduras. |
Table 1. Species of endophytic fungi associated with roots of banana and plantain in plantations on different locations, Cuba.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Clone</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>F. equiseti (Corda) Sacc.</td>
<td>Gran enano</td>
<td>UBPC 14 La Cuba, Ciego de Avila</td>
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<td>UBPC 1 La Cuba, Ciego de Avila</td>
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<td>Gran enano</td>
<td>UBPC 1 La Cuba, Ciego de Avila</td>
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<tr>
<td>2.4</td>
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<td>Gran enano</td>
<td>UBPC 1 La Cuba, Ciego de Avila</td>
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<td>Sola, Camagüey</td>
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<td>Gran enano</td>
<td>Sola, Camagüey</td>
</tr>
<tr>
<td>6.1</td>
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<td>6.3</td>
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<td>8.1</td>
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<td>8.2</td>
<td>Basidicarp</td>
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<td>Fhia-03</td>
<td>Lenin, Matanzas</td>
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<td>Fhia-03</td>
<td>Lenin, Matanzas</td>
</tr>
<tr>
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<td>Gran enano</td>
<td>Lenin, Campo 48, Matanzas</td>
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<tr>
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<td>Gran enano</td>
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<td>CPA Niceto Pérez, Güira La Habana</td>
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<td>Rpto. Hnos. Cruz, Pinar del Río</td>
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<td>Burro</td>
<td>San Juan y Martínez, Pinar del Río</td>
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<td>Cavendish enano</td>
<td>Cofa, Boyeros, La Habana</td>
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<td>CCS Pedro Lantigua, Bauta, La Habana</td>
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<td>31</td>
<td>F. oxysporum Schlecht.</td>
<td>Consejo de Estado, Plaza, La Habana</td>
<td></td>
</tr>
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<td>Robusta</td>
<td>Fca. Covín, Caimito, La Habana</td>
</tr>
</tbody>
</table>

Figure 1. Roots of banana with necrosis caused by nematode attack.

Figure 2. Cylindrocarpon musae. Macroconidia and chlamydospores.

Booth and Stover (1871) reported the presence of C. musae associated with root necrosis in banana in Costa Rica, however the fungus did not have the parasitic capacity to cause lesions on healthy roots. Other species of Cylindrocarpon are important pathogens of plants. For example C. destructans causes root necrosis and death in pine (Pinus sp.) (Chakravarty and Unestam 1987).

Recently there have been bioassays of artificial inoculation of C. musae alone or of C. musae in co-inoculation with R. similis. The studies provided important information on the effects of the pathogenicity of these species on root necrosis in the crop.

None of the sites yielded species of Cylindrocladium or Zythia as reported by Loridat (1989), Mourichon (1993) and Risède (1994). These species have been associated with necrosis of banana in Martinique and Guadeloupe, and latterly in Cameroon (Abadie 1998, pers. comm.) and Côte d’Ivoire (Kobenan 1991).

Conclusions

1. F. oxysporum and C. musae were the species most frequently associated with necrosis caused by nematodes in plantations of banana and plantain in different parts of Cuba. F. equiseti and Rhizoctonia spp. were found less frequently.

2. F. oxysporum was the most frequent isolate accounting for 45.6% total isolates, followed by C. musae.
Cylindrocladium and Zythia spp., reported in other countries to be associated with root necrosis, were never found.

References

Agronomy

Effects of mycorrhization on the development of two cultivars of micropropagated banana

M.C. Jaizme-Vega, M. Esquivel Delamo, P. Tenoury Domínguez and A.S. Rodríguez Romero

The likelihood of using arbuscular mycorrhiza (AM) in crop production systems is increasingly more realistic and studies have increased considerably in the last few years. Banana (Musa AAA) in its early stages of development is readily colonized by mycorrhiza and is moderately (40-50%) dependent on them (Jaizme-Vega et al. 1998). Mycorrhization in vivo has resulted in large increases in the growth and nutrition of this species (Lin and Chang 1987, Rizzardi 1990, Declerk et al. 1995, Jaizme-Vega and Azcón 1995) including in the presence of standard fertilization regimes in commercial nurseries (Tenoury 1996, Sosa Hernández 1997), with favourable effects on plant behaviour when confronted with various soil-borne pathogens such as Meloidogyne incognita (Jaizme-Vega et al. 1997), Pratylenchus goodeyi (Jaizme-Vega and Pinochet 1997) and Fusarium oxysporum f.sp. cubense (Jaizme-Vega et al. 1998). These results demonstrate the advantages of applying inoculum of fungal AMs during root production and acclimatization of micropropagated banana plants, which gives rise to plants that are well developed and have an increased tolerance to attack by soil-borne pathogens. However, at present there is no information on the effects of such symbiotic fungi on the banana plant during the later stages of development and with fertilizer regimes similar to those practised in commercial crops.

Therefore, the sequential effects of early mycorrhization on the growth of micropropagated banana plants were studied from the earliest stages of development until nine months after transplanting to the field in microplots.

Materials and methods

Host plant
Micropropagated material of the two most widespread commercial cultivars of banana Musa acuminata Colla AAA, cvs. ‘Grande naine’ and ‘Gruesa’ (a local selection of ‘Dwarf Cavendish’) was used.

Rooting stage
Inoculation with AM fungi
Mycorrhization was done during hardening off. Inoculum comprised a homogenous mixture of rhizosphere soil, spores and rootlets of the host plant.

Each cultivar was inoculated with one of two AM fungi, each with 1500 g inoculum per tray (capacity of tray 24 kg) with the following isolates:
- Glomus intraradices Schenck & Smith, from stock collection, multiplied on sorghum, and giving 68% colonization;
- Glomus manihotis Howeler, Sieverding and Schenck, from stock collection, multiplied on tomato, and giving 70% colonization.

At inoculation, plants were 10 cm ± 2 cm and had approximately three developed leaves. Inoculation was in polyethylene (PE) trays (40 x 60 cm, H x L), each tray containing one cultivar/fungus combination with an additional two control trays with non-inoculated plants, one tray per cultivar. Thus there was a total of six trays each with 35 plants.

The substrate comprised a steam-sterilized mixture of dark-coloured volcanic soil and amended peat (TKS1®, Instant, Floragard, GmbH) in a proportion of 5:2:1. This phase lasted six weeks in a glasshouse and under a tunnel of black mesh for acclimatization. Irrigation was with distilled water according to the needs of the plants.

Nursery phase
At the end of root production and before transplanting to individual containers, 10 plants of each treatment/cultivar combination were selected and the effects

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evaluated of inoculation with mycorrhiza on plant development, the mycorrhizal dependency under the fixed conditions of fertilizer inputs, and the extent of colonization by the AM fungi.

Parameters relevant to the growth of the plant in general were evaluated at each stage of the investigation as follows: fresh weight (g) of roots and aerial parts, dry weight (g) of aerial parts, length and diameter (cm) of pseudostem, leaf numbers and area (cm²). Leaf area was calculated with an area meter Li-COR, Inc. Lincoln, Nebraska, USA, model Li-3100.

The relative mycorrhizal dependency (RMD), defined by Gerdeman (1975) as the degree of mycorrhization needed by plants to produce the maximum growth or yield depending on fertility of the soil, was calculated according to the formula proposed by Plenchette et al. (1983) as the numerical expression of this concept:

\[
RMD = \frac{\text{DW of plant with AM} - \text{DW of plant without AM}}{\text{DW of plants without AM}} \times 100
\]

Infection by the mycorrhiza was confirmed by observation with a light microscope. Root samples were bleached with 10% KOH and then stained with 0.05% trypan blue in lactic acid as described by Phillips and Hayman (1970) and modified by Koske and Gemma (1989). Percentage root colonization was determined on 20 1-cm sections of stained root, mounted on slides and examined with a light microscope as described by Brundett et al. (1985).

Once the determinations were complete 20 plants of each treatment were transferred to 2 L PE bags containing a substrate comprising equal volumes (1:1:1) of steam sterilized soil (‘pirócn’) and enriched peat (TKS1®). This phase took 14 weeks in glasshouse conditions at temperatures of 27-32°C, and a relative humidity of 70-80%.

Fertilization was according to the fertilizer regime of a commercial banana nursery. Plants were fertilized twice weekly (100 cc/plant) on alternate days. One of the fertilizer applications was with (NO₃)₂Ca (3 g/L) and NO₃H (0.4 cc/L), and the other application was with SO₄K₂ (3 g/L) and PO₄H₃ (0.2 cc/L). The days on which fertilizer was not applied alternated with irrigation with running water according to the needs of the crop. Plants received a weekly foliar application of micronutrients consisting of 3% Wuxal® Super AA 8-8-6 (Argos Shering, Agrevo, S.A., Valencia, Spain).

**Microplot phase**

After growth for 3.5 months, plants were transferred to larger containers, and buried in a plot within the boundaries of the ICIA estate situated 300 masl. The site was chosen on the basis of aspect, climatic conditions and as an area marginal for this crop. Prior to this, and as with the first transplanting, 10 plants per cultivar and treatment were evaluated for the effects of the AM fungi, that is the extent of root infection by mycorrhiza and mycorrhizal dependency.

For this last phase of the trial, PE pots 35 cm diameter and 50 L volume were selected and filled with non-sterilized medium of the same materials and in the same proportions as described for the previous transplanting (1:1:1), and amended with 1.5 g/L of slow release fertilizer (Osmocote 17:10:10, Scotts, O.M. Tarragona). Once in position in their new pots (10 per cultivar and treatment), the plants were placed amongst other similarly sized pots previously buried up to the upper edge of pot, in the trial plot. Plants were fertilized weekly (1 L/plant), via the localized irrigation system, with the two combinations of fertilizer treatment described previously for banana plants after the first transplanting. Foliar fertilizers were applied fortnightly. The days on which fertilizers were not applied, plants were irrigated according to the needs of the plants.

Plants remained in position for nine months. The trial was then terminated and the effects of symbiosis on development of the banana plants evaluated.

The following experimental variables were studied: fresh weight of roots and aerial parts, numbers of suckers, numbers of leaves, leaf area, N, P and K content, and dependency for mycorrhiza.

On completion of the foliar analyses, the samples were transferred to a heater for 24 hours at 70°C after which nitrogen, phosphorous and potassium contents were determined. For N determination, the sample was mineralized “via humid process”, P was determined colourimetrically and K by spectrophotometry of atomic absorption.

Data were analyzed by means of ANOVA (Systat). Means were compared by Fisher’s test of least significant differences (LSD) using the statistical package Systat version 5.0 (SPSS Inc., Chicago, USA).

**Results and discussion**

By completion of the rooting stage, both cultivars showed a positive response to...
the two AM fungi used for inoculation (Tables 1a and 2a). In this phase, the relative mycorrhizal dependency (RMD) of both cultivars to Glomus manihotis and Glomus intraradices were the highest throughout the trial and were 35% and 50% respectively. In this first phase the percentage colonization by the two inoculated AM fungi was similar for the two inoculated cultivars completing this phase of the trial with averages of 40% for both AM fungi on ‘Grande naine’, and 30% and 20% respectively for Glomus manihotis and Glomus intraradices on cv. ‘Gruesa’ (Tables 1b and 2b).

Following transplanting, the positive effect of the AM fungi on plant development was maintained for 3.5 months after mycorrhization. For inoculated plants of both cultivars, the majority of experimental variables were significantly different for both cultivars in comparison with the controls (Tables 1b and 2b). The development of RMD was similar for both cultivars completing this phase of the trial with averages of 40% for both AM fungi on ‘Grande naine’, and 30% and 20% respectively for Glomus manihotis and Glomus intraradices on cv. ‘Gruesa’ (Tables 1b and 2b). Root colonization of banana plants by mycorrhizae tended to differ depending on the cultivar. Thus, roots of ‘Grande naine’ inoculated with G. manihotis had twice the mycorrhiza infection in comparison with the beginning of the study, similar results being maintained on roots colonized by G. intraradices. However with plants of cv. ‘Gruesa’ no changes in root colonization were observed in comparison to the first transplanting. During the trial, from 14 weeks onwards 15% root infection by contaminant AM fungi was noted in control plants of both cultivars (Tables 1b and 2b) but without significant effects on plant development. These endophytes are able to disperse in irrigation water or by uncontrolled contamination in the nursery containing the plants. These data confirm those already published on the benefits of early mycorrhization of plants in the first phases of development of this crop (Declerck et al. 1995, 1996, Sosa-Hernández 1997, Jaizme-Vega et al. 1997, 1998).

The results of the second phase of the trial in which the effects of the AM fungi on mycorrhiza-treated plants in the in vivo phase for three months and transplanted to non-sterile medium, showed that after nine months in microplot conditions and a standard fertilizer regime, banana plants inoculated with Glomus intraradices usually, particularly with cv. ‘Gruesa’, showed a beneficial effect of

---

**Table 1.** Effect of Glomus manihotis and G. intraradices on the development, colonization and mycorrhizal dependency of micropropagated banana cv. ‘Grande naine’ at a) 6 weeks after inoculation, b) 14 weeks after inoculation and c) 9 months after transplanting to microplots.

<table>
<thead>
<tr>
<th>Root Aerial parts</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Pseudostem Diameter</th>
<th>No. leaves</th>
<th>Leaf area (cm²)</th>
<th>Colonization (%)</th>
<th>RMD**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glomus manihotis</strong></td>
<td>6.4 a</td>
<td>17.5 a</td>
<td>1.1 a</td>
<td>12.2 a</td>
<td>6.3 a</td>
<td>261 a</td>
<td>51</td>
</tr>
<tr>
<td><strong>G. intraradices</strong></td>
<td>7.7 a</td>
<td>16.2 a</td>
<td>1.0 a</td>
<td>12.1 a</td>
<td>6.0 a</td>
<td>269 a</td>
<td>46</td>
</tr>
</tbody>
</table>

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<th>Dry weight (g)</th>
<th>Pseudostem Diameter</th>
<th>No. leaves</th>
<th>Leaf area (cm²)</th>
<th>Colonization (%)</th>
<th>RMD**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G. manihotis</strong></td>
<td>6.8 a</td>
<td>13.7 a</td>
<td>1.0 a</td>
<td>12.1 a</td>
<td>6.0 a</td>
<td>269 a</td>
<td>46</td>
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<td>6.0 a</td>
<td>269 a</td>
<td>46</td>
</tr>
</tbody>
</table>

---

**Table 2.** Effect of Glomus manihotis and G. intraradices on the development, colonization and mycorrhizal dependency of micropropagated banana cv. ‘Gruesa’ at: a) 6 weeks after inoculation, b) 14 weeks after inoculation, and c) 9 months after transplanting to microplots.

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<th>Leaf area (cm²)</th>
<th>Colonization (%)</th>
<th>RMD**</th>
</tr>
</thead>
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<td>12.1 a</td>
<td>6.0 a</td>
<td>269 a</td>
<td>46</td>
</tr>
</tbody>
</table>

---

* Means of 10 replicates. Within each column, differences between numbers followed by the same letter are not statistically different by Fisher’s test (P ≤ 0.05).

** RMD: relative mycorrhizal dependency.

---

* Means of 10 replicates. Within each column, differences between numbers followed by the same letter are not statistically different by Fisher’s test (P ≤ 0.05).

** RMD: relative mycorrhizal dependency.
symbiosis on plant development, with RMDs of approximately 40%. These values are considered to be relatively high for the conditions of the trial (Tables 1c and 2c); moreover there was an increase in the other experimental variables. However, data on macronutrients (N, P and K) although noticeably higher, did not differ statistically (Tables 1c and 2c). This lack of response in nutrient content of aerial parts can be interpreted as typical for a mycorrhiza-treated plant receiving soluble fertilizer.

Plants of cultivar ‘Grande naine’ showed a smaller response to the AM fungi after the microplot phase, plants inoculated with G. manihotis showing a develop- mental and nutritional state equal or slightly less than control plants.

At the end of this phase, root colonization by both Glomus species was relatively important in both cultivars (greater than 79%). Attention is drawn to the high level of colonization of roots of control plants. This part of the trial used non-sterilized substrate which, together with other conditions in the trial, explained the data.

In conclusion, in general and particularly in the last phase of the trial, we can confirm that, at the later stages of the crop, this biotechnological resource showed promise for the improvement of production.

Acknowledgements
The authors thank Ana Rosa Socorro Monzón, head of the Laboratory for soils and irrigation, ICIA, for the leaf analyses.

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References

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Agronomy | Use of cover crops

Arachis pintoi: a cover crop for bananas? Advantages and disadvantages as regards nematology

P. Quénéhervé, Y. Bertin and C. Chabrier

The legume Arachis pintoi L. (the perennial, wild or pinto peanut) has been used for many years as a cover crop in many tropical countries, notably Central America (Kerridge 1993). Its response to nematodes is still barely documented. Possible resistance of cv. Amarillo to Meloidogyne spp. in Australia has been mentioned (Cook et al. 1990). In Mexico a noticeable reduc- tion in Meloidogyne attacks on tomatoes was observed in an intercropping experiment (Marban Mendoza et al. 1992). In Costa Rica, a field experiment showed that Arachis pintoi is a good host for Radopholus similis (Cobb 1993, Thorne 1949) with a concomitant mean infection rate of about 30 individuals per g of root (Araya 1996). Also in Costa Rica, trials carried out with bananas and plantains have shown a beneficial effect of wild peanut used as a cover crop in reducing the density of Radopholus similis on neighbouring banana plants (Vargas 1998). Lastly, in 1999, Jonathan et al. showed from an artificial inoculation experiment that the legume Arachis pintoi is not a host for certain species of Meloidogyne Goeldi 1892 (M. incognita, M. arenaria, M. javanica) nor for Rotylenchus reniformis Lindford & Oliveira 1940.

Before experimenting with, and perhaps recommending the use of Arachis pintoi as a possible cover crop for bananas, we wanted to check its behaviour towards nematodes of banana in Martinique. A controlled inoculation trial
using the main species present (Radopholus similis, Pratylenchus coffeae, Hoplolaimus seinhorsti, Meloidogyne incognita) together with Meloidogyne mayaguensis, a species which is very pathogenic in Martinique although not yet observed on bananas, was therefore carried out in a controlled environment chamber at the IRD Nematology laboratory before attempting any field experiments.

Materials and methods

Seeds of Arachis pintoi cv. Amarillo from Costa Rica were inoculated by coating at the moment of sowing with their symbiotic bacterium Rhizobium sp. These seeds were then grown in 237 cm³ PVC culture tubes filled with sterile soil (steam sterilization for 1 h at 100°C). The substrate was a volcanic andosol of pH 6.2, with 7.3% organic matter and a cation exchange capacity of 10.3 meq per 100g soil. The experiment was carried out in a controlled environment chamber with eight replicates, using a 14-h photoperiod and a temperature in the light of 27±1°C, and in the dark of 22±1°C, daily watering and weekly application of a Hoagland nutrient solution.

Four weeks after sowing and development of the peanut, the five nematode species, meanwhile grown in the laboratory (Radopholus similis, Pratylenchus coffeae, Hoplolaimus seinhorsti, Meloidogyne incognita and Meloidogyne mayaguensis) were individually inoculated at a rate of 400 individuals per plant. The infestation of the root system was checked 45 days later after extraction of the nematodes from the roots by spraying (Seinhorst 1950). The nematode densities were then expressed as numbers of nematodes per root system and per g of dry root (after oven-drying at 60°C for 24h).

Results and discussion

The results of this experiment (Table 1) show that at 45 days only three species of nematode are supported: R. similis, H. seinhorsti and P. coffeae. The inoculation of the different species of nematode has had no effect on the growth either of shoots or roots of the peanut, which therefore appears, over this short period of time, to be tolerant of attack by these nematodes.

Arachis pintoi was unable to maintain or permit the multiplication of the two species of Meloidogyne, M. incognita and M. mayaguensis. This result confirms and completes for M. mayaguensis earlier results on the inability of this peanut to act as host to the main species of root-knot nematodes, with the exception of M. hapla (Jonathan et al. 1999).

Arachis pintoi does however act as host to three other species, and according to the criteria applied to banana weeds (Quénéhervé et al. 2002), one might say that it is a bad host for R. similis but a very good host for H. seinhorsti and P. coffeae. The hosting capacity of Arachis pintoi to R. similis already observed (Araya 1996) is thus confirmed, but also (which is new) its great susceptibility to P. coffeae and H. seinhorsti, two species of nematodes whose pathogenicity to bananas was demonstrated for one (P. coffeae) and seems very likely for the other.

These results may be compared with others from the field under conditions of natural infestation. In fact, root produc-

Table 1. Results of nematode counts and Arachis pintoi weighings 45 days after inoculation.

<table>
<thead>
<tr>
<th>Species</th>
<th>No./g roots</th>
<th>Roots (mg)</th>
<th>Shoot (mg)</th>
<th>Host quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>260 ± 10</td>
<td>1470 ± 140</td>
<td>-</td>
</tr>
<tr>
<td>Hoplolaimus seinhorsti</td>
<td>382 ± 132</td>
<td>240 ± 40</td>
<td>1880 ± 110</td>
<td>***</td>
</tr>
<tr>
<td>Pratylenchus coffeae</td>
<td>2918 ± 447</td>
<td>240 ± 30</td>
<td>1630 ± 350</td>
<td>***</td>
</tr>
<tr>
<td>Radopholus similis</td>
<td>112 ± 95</td>
<td>250 ± 50</td>
<td>1820 ± 50</td>
<td>*</td>
</tr>
<tr>
<td>Meloidogyne mayaguensis</td>
<td>0</td>
<td>240 ± 60</td>
<td>1600 ± 300</td>
<td>NH</td>
</tr>
<tr>
<td>Meloidogyne incognita</td>
<td>0</td>
<td>270 ± 30</td>
<td>2010 ± 180</td>
<td>NH</td>
</tr>
</tbody>
</table>

ANOVA NS NS

1 Very good host = ***; Good host = **; Poor host = *; Non-host = NH

For many years agronomists have sought plants useful as fallows of short, medium or long term, or as cover crops which can, inter alia, reduce parasitic pressure (for example by nematodes) and also reduce the effects of weeds, improve soil fertility and limit erosion (Terisien and Melin 1989). In the Caribbean zone, two species have been used for their activity against nematodes, with both advantages and disadvantages: the forage grass Digitaria decumbens and the forage legume Mucuna pruriens cv. utiliss, of African origin.

Each of these plants has its value according to the cropping system being considered. Digitaria decumbens fits into long-term rotations combining livestock and field vegetable growing, as practised on the vertisols in the south of Martinique. Mucuna pruriens, widely grown in the southeastern United States and in Africa, can also find a place in Martinique as a short-term intercrop or in certain intensive vegetable systems to control nematodes, particularly Meloidogyne spp. (Quénéhervé et al. 1998).

This third plant, Arachis pintoi, recently introduced by CIRAD-FLHOR into Martinique, seems to offer certain advantages, but also possesses disadvantages:

- advantages: commercially available seed, propagation by seed, or vegetative; "non-host" plant to several nematode species including Meloidogyne spp.; suitable as a cover crop; supplies nitrogen (about 60 kg/ha/year).
• disadvantages: plants are host to serious migratory endoparasites, including R. similis and P. coffeae; slow to establish; requires inoculation with a specific associated bacterium.

The introduction and use of Arachis pintoi as a cover crop for bananas could therefore take place under certain conditions:

- in the absence of the nematodes R. similis and P. coffeae, which would limit its use immediately after banana or another crop infested by P. coffeae, such as yam or dasheen;
- after crop rotation but in the presence of Meloidogyne spp. so as to reduce the infestation potential of these root-knot nematodes before replanting with tissue-cultured banana plants.

This plant could also find a place in Martinique and elsewhere in the West Indies in various other ecosystems, which remain to be experimented with:

- in orchards such as citrus and especially guavas which suffer serious attacks from M. mayaguensis in the West Indies (Quénéhervé et al. 2001);
- as a fallow crop or intercropped cover crop for vegetables.

References

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Agronomy

Micronutrient studies

Dynamics of boron in a soil cultivated with plantain (Musa AAB cv. ‘Dominico hartón’) in the Quindío, Colombia

M.M. Bolaños Benavides and A. Garcia Alzate

Boron (B) is the only non-metallic element of the six essential micronutrients; it has a constant valence of +3, and has the smallest ionic radius. It is found mainly in sedimentary rocks. Of the igneous rocks it is most abundant in granites, in the form of borosilicates, with tourmaline (3-4% boron) the most common of the minerals. It is found in soil in four states: a) forming part of the crystalline structure of minerals; b) adsorbed or bound by soil colloids; c) as an anion in the soil solution and d) associated with soil organic matter (Bonilla et al. 1994).

The total content of boron in soils varies from 2 to 200 ppm most of which is not taken up by plants. Compared with other micronutrients, boron has several special features, thus in soil solution it is always found in combination with oxygen, behaving as an anion (borate) in all reactions. The borate anion is highly mobile and hence is easily lost by leaching. The available boron in soil can be considered as belonging to a cycle where a small amount originates from tourmaline and a large part from soil organic matter.

Organic material is decomposed by microorganisms which liberate available boron to the soil solution, from whence it is taken up by plants; part can be washed out by percolating water, and a small part is fixed or bound by clays (Berger and Pratt, cited by Bonilla et al. 1994).

The multiple functions involving boron in plant metabolism include the following: it affects, inter alia, the processes of flowering and fruiting, germination of pollen grains, cell division, cell wall synthesis and the metabolism of nitrogen, carbohydrates and pectic substances. These substances are reported to increase in plants that are deficient in boron (Rajaratnam and Lowry 1974).

Another function of boron is the absorption of water by protoplasm and the absorption of mineral salts. The main function of boron is reported to facilitate the transport of highly polar sugar molecules across the cell wall. Boron is a constituent of cell membranes and is immobile in the plant; therefore any boron deficiency is immediately reflected in a change in the metabolism of carbohydrates (which accumulate in leaves). This condition could be the cause of almost all the remaining functions attributed to boron (Gómez and Seinhorst J.W. 1950. De betekenis van de toestand van de grond voor het optreden van aanstorting door het stengelaattje (Ditylenchus dipsaci) (Kühn) Filipjev). Tijdschr. Plziekt. 5: 291-349.

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Leguizamón (1975). In spite of the important advances in mineral nutrition, the role of boron in plant metabolism still raises many questions.

At present, in the coffee production area of central Colombia, many plantain crops show symptoms associated with boron deficiency. According to León et al. (1985) ten examples of boron deficiency were reported in the country. In the present study we tried to obtain a sound foundation in order to deal more clearly with the problems above. The objective was to determine the importance of boron in the cultivation of plantain (Musa AAB cv. Dominico hartón) in the Quindío, and to study the dynamics over a period of ten years, in a soil fertilized with the major elements.

### Materials and methods

The study plot was located at the Experimental station El Agrado, District of Montenegro, Department of Quindío, Colombia. The station was 1320 m above sea level, with an average rainfall of 2000 mm/annum, a mean annual temperature of 22°C and a relative humidity of 76%.

According to the classification of Holdridge, the ecosystem corresponds to premontane humid woodland. The soils are derived from volcanic ash (andisols) and have an average natural fertility, a medium to heavy texture, a low moisture retention capacity, and are leached and susceptible to erosion.

For the study the soils were taken for analysis from 2 May 1990 until 2 March 2000. Soil samples, replicated five times, were taken every two years. Rainfall data was analyzed for the same period.

Samples were analyzed for pH, organic matter content, exchangeable calcium, phosphorus (P), magnesium (Mg), potassium (K) and boron (B). The analytical methods are described in Table 1. The data were analyzed to determine the correlation between: boron-weight of bunch (for each production cycle), boron-potassium, boron-calcium, boron-percentage soil organic matter, and boron-pH. The relationships between Ca/Mg, Mg/K, Ca/K and (Ca+Mg)/K were also analyzed.

### Results and discussion

The data obtained from soil analyses, averaged over the five replicates, changed over the years as shown in Table 2.

#### Boron content

As can be seen from the chemical analyses during the 10 years of the study, boron content declined considerably from levels sufficient for cultivation of plantain, according to Buriticá (1985) from 0.4 ppm to 0.01 ppm boron, a value which gave rise to deficiencies. However, one must consider the edaphic cycle of boron which determines its concentration in soil solution and hence the availability for uptake by plants (Mengel 1980).

#### Relationship between pH and boron content

As can be seen from Table 3, boron showed a direct and close correlation with pH, hence this is in a range optimum for absorption of boron; fixation of this microelement to hydroxides of Fe and Al, as is with clays, increases with pH being a maximum between pH 8 and 9 and a minimum at about pH 5 (Lora 1994). According to Domeíquez (1988) the increase in pH reduces the availability of boron but this does not become evident until more than pH 6 which did not occur after the start of the experiment.

According to Marschner (1986) the availability of boron to plants decreases with increased soil pH, as happens in calcareous soils or in soils with high clay content, presumably as a result of the formation and absorption of B(OH)₄⁻.

In agreement with the soil chemical analyses, the pH value (5.1-6.08) fluctuated in the range sufficient for the micronutrient to be available. This explains why symptoms of boron deficiency only became evident in the last years. The explanation for the correlation between boron content and pH, are based on the following:

- Profoundly influences many biological processes in soil,
- Affects the availability of micronutrients,
- Alters the absorption of an element and its effect on microbial activity,
- Results in changes in the ability of roots to absorb or transport ions once they have been taken up,
- Causes variations in the stability of soluble and insoluble organic complexes,
- Changes the solubility of antagonistic ions and changes conditions in the rhizosphere.

### Relations between nutrients

The results also show a close inverse correlation between K and boron (Table 3) explicable because the K content over the years had reached levels greater than 0.3 meq/100 g soil, which, according to Gómez and Leguizamón (1975) can induce boron deficiency.

The potassium-boron interaction does not appear to follow a particular pattern. Revé and Shive (1944) cited by Domínez (1988), demonstrated that in a boron-rich medium, absorption of boron increased as the soil became enriched with K but, in contrast, when boron levels in the medium are low, boron deficiency becomes worse as K increases. The direction of the interaction between K and boron appears to depend on the amount of boron in soil solution. The trend in this study showed that increasing applica-

### Table 1. Methods of chemical analysis of soils.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Analytical methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Potentiometer, ratio 1:2.5</td>
</tr>
<tr>
<td>EA (exchangeable acidity)</td>
<td>KCl 1N</td>
</tr>
<tr>
<td>MO</td>
<td>Walkley-Black</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>Bray II</td>
</tr>
<tr>
<td>Exchangeable bases</td>
<td>Ammonium acetate (1N) and neutral (pH 7)</td>
</tr>
</tbody>
</table>

### Table 2. Changes in chemical properties of soil under study (1990–2000).

<table>
<thead>
<tr>
<th>Year</th>
<th>pH</th>
<th>MO (%)</th>
<th>K (meq/100 g)</th>
<th>Ca (meq/100 g)</th>
<th>Mg (meq/100 g)</th>
<th>P (ppm)</th>
<th>B (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>6.08</td>
<td>3.79</td>
<td>0.95</td>
<td>5.2</td>
<td>0.93</td>
<td>22</td>
<td>0.40</td>
</tr>
<tr>
<td>1993</td>
<td>5.18</td>
<td>3.66</td>
<td>0.69</td>
<td>4.4</td>
<td>1.03</td>
<td>71.6</td>
<td>0.12</td>
</tr>
<tr>
<td>1995</td>
<td>5.72</td>
<td>3.72</td>
<td>1.22</td>
<td>2.8</td>
<td>0.64</td>
<td>34.6</td>
<td>0.19</td>
</tr>
<tr>
<td>1997</td>
<td>5.78</td>
<td>4.80</td>
<td>1.30</td>
<td>3.8</td>
<td>0.94</td>
<td>61.0</td>
<td>0.06</td>
</tr>
<tr>
<td>2000</td>
<td>5.10</td>
<td>4.80</td>
<td>1.79</td>
<td>6.0</td>
<td>0.60</td>
<td>29.0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### Table 3. Correlations between soil boron, pH, K, Ca, P and weight of bunches (WB) in kg.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH – B</td>
<td>0.82</td>
</tr>
<tr>
<td>K – B</td>
<td>-1</td>
</tr>
<tr>
<td>Ca – B</td>
<td>0.8</td>
</tr>
<tr>
<td>P – B</td>
<td>-0.86</td>
</tr>
<tr>
<td>WB – B</td>
<td>1</td>
</tr>
</tbody>
</table>
tions of K resulted in a small reduction in boron availability.

When boron interacts with other elements, it is necessary to consider the possibility of nutritional imbalances in soil, given that this implies an antagonism that affects the plant directly, as one or other elements would not then be available. Such is the situation with potassium that is absorbed in smaller amounts when boron content is very low.

As regards calcium, the levels increase when boron is deficient. In the soil under study, where we did not find a high availability of Ca, this could favour boron uptake. However, the interaction calcium-boron has been studied with rather high concentrations, and with the relationship of Ca/boron in the plant. Revé and Shive (1944) cited by Domínguez (1988), indicated that high concentrations of calcium exacerbate symptoms of boron deficiency in tomato. The toxicity of boron, in a medium with too much of the element, can on the other hand have a reduced increase in the amount of calcium in the medium. It is possible that with all the preceding, boron deficiencies in the cultivation of plantain studied, only became apparent in the last years (1999-2000) even when the low levels of this element were present from 1993.

Table 3 shows an inverse correlation of P to boron. According to studies by Robertson and Loughman (1974), it was evident that there was a clear reduction in the uptake of phosphorus in boron-deficient plants. This idea is based on the role of boron as a stimulant of the mineralisation of organic material leads to a release of assimilable boron. Berger and Truog (1945) cited by Domínguez (1988), obtained a positive relationship between assimilable boron (water soluble boron) and the organic matter content of soil. More recently, Olsen and Berger (1946) cited by Domínguez (1988), demonstrated that mineralization of organic material leads to a release of assimilable boron.

According to the relationships obtained between the different cations (Table 4) and later comparisons with the critical levels, K was never seen to be deficient, which is explained by the large quantities of potassium containing fertilizers that were applied over the years, as well as by the recycling that occurs with this element in residues from the harvest of the plantain. According to Belalcázar (1991), cultivation of plantain removes a large percentage of elements such as potassium (76.02%) and calcium (13.62%), followed by nitrogen, magnesium and phosphorus. Those with the higher percentage that are removed are nitrogen (25.55%) followed by magnesium (19.80%) and phosphorus (19.09%), whilst those that are reincorporated or recycled in higher amounts are calcium (94.47%) and potassium (89.77%).

With reference to magnesium, amongst the factors that gave rise to the deficiency in this macronutrient, were unsuitable relationships with the other bases in the soil, mainly potassium (Table 4). The relationship Mg/K appeared to be unbalanced, explaining a deficiency of Mg. Therefore, the high levels of K function in a manner antagonistic to Mg, implying a low absorption for this element. The losses of magnesium in soil are greater when added with potassium fertilizers. Many authors consider that a soil to be low in magnesium when it has less than 1.0 meq/100 g is present, whilst others classify soils as poor in magnesium when there is less than 1.5 or even 2.0 meq/100 g (Suárez and Carrillo 1984).

Intensive and continual fertilisation with K, as employed in this zone, possibly contributes to Mg deficiency, resulting in an imbalance in the relationship Mg/K and as a result an inhibition of Mg uptake. It should be noted that in the zone where this study took place, it is common to come across crops with symptoms of magnesium deficiency. In accordance with Fried and Dean (1952) nutritional deficiencies resulting from an imbalance can be corrected by a programme of balanced fertilisation.

### Soil organic matter and boron in soil

In accordance with the results of this study there was no correlation between organic matter and boron, however, it should be noted that various authors (Gómez and Leguizamón 1975) claim that in mineral soils rich in organic matter, boron deficiency is rarely seen because soil organic matter is a major source of boron. Similarly, Berger and Truog (1945) cited by Domínguez (1988), obtained a positive relationship between assimilable boron (water soluble boron) and the organic matter content of soil. More recently, Olsen and Berger (1946) cited by Domínguez (1988), demonstrated that mineralization of organic material leads to a release of assimilable boron.

On the other hand, boron absorbed on organic and inorganic soil colloids constitutes a reserve that maintains the concentration of boron in solution; this helps to replenish the demand by crops and reduces losses by washing. Furthermore, soils with higher organic matter content

---

### Table 4. Relationships between cations in the experimental soil (1990-2000).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg/K</td>
<td>1.58</td>
<td>1.93</td>
<td>0.87</td>
<td>0.73</td>
<td>1.00</td>
</tr>
<tr>
<td>Ca/Mg</td>
<td>5.72</td>
<td>4.14</td>
<td>5.00</td>
<td>4.88</td>
<td>5.00</td>
</tr>
<tr>
<td>Ca/K</td>
<td>8.97</td>
<td>7.96</td>
<td>5.37</td>
<td>3.58</td>
<td>6.66</td>
</tr>
<tr>
<td>(Ca+Mg)/K</td>
<td>10.50</td>
<td>9.88</td>
<td>6.26</td>
<td>4.33</td>
<td>7.66</td>
</tr>
</tbody>
</table>

---

![Figure 1](image1.png) **Figure 1.** Production of plantain in relation to boron content in soil.

![Figure 2](image2.png) **Figure 2.** Boron content of soil vs. rainfall.
have higher concentrations of boron, since an important fraction of soil boron comes from soil organic matter.

**Production of plantain and soil boron**

There was a close and direct correlation between yield and boron (Table 3). This is explicable as a result of the chemical degradation of the soil, as shown in Table 2. The gradual loss of the micronutrient boron, drastically affects the filling of the fruit (Figure 1) to the point where the young fruits become deformed, mature prematurely and size is reduced, hence yield declines and they are difficult to market. Clearly productive capacity of plantain is harmed. The reduction in yield can also be associated with the regulation of uptake and translocation of boron by the plants, which is more limited in comparison with other minerals.

At the same time, the low quantity and quality of production could be caused by an early boron deficiency, which checks growth of the apices and restricts cell elongation (Lovatt et al. 1981, Robertson and Loughman, 1974b) and cell division (Cohen and Lepper 1977).

According to Leguizamón (1975), in many situations the affected developing bunches do not produce a yield and in this situation, bunches are small and deformed.

From the above it can be concluded that boron is a nutrient fundamental to good production as well as fruit quality and in quantity.

**Rainfall and boron**

Boron content tends to decline as a result of the high rainfall which occurs in the area of the study as shown in Figure 2; this, linked to the sandy loam type texture, and the definite mobility of the boron anion, gave rise to an increased rate in leaching of boron. Thus, the nutrient should be applied in a more finely divided form.

This result was in agreement with Marschner (1986) who suggested that under conditions of high rainfall boron is washed out as B(OH)₃⁻.

**Boron and the physiology of the plant**

A general aspect of boron deficiency is the poor development of meristematic tissues, as found at the tips of young roots and in the buds. With boron deficiency, irregularities in development are the first symptoms (Domínguez 1988). This check to the growth of the root tips possibly contributes to one of the main problems of the cultivation of plantain, which harms the plant. Primavessi (2000) confirmed that the addition of boron aids root growth, and that if this continues in the coat with organic matter and roots do not want to penetrate the soil, there may not be sufficient boron.

Boron deficiency in plants is not easily identified except by leaf or soil analysis. This is important in the cultivation of plantain because the micronutrient plays a key role in the transport of sugars, as a result of the transformation of boron-sugar complexes (Marschner 1986) and therefore affects the filling of the fruits; in such situations, a deficiency directly and adversely affects the quality and quantity of the plantain harvest.

**Conclusions**

- Boron is crucial for optimal yields of plantain, as well as for the quality and quantity of fruit. This was confirmed by the correlation between bunch weight and boron.
- The availability of boron in soil solution is closely linked to rainfall and the loose sandy loam texture of the soil and the definite mobility of the borate anion.
- In the experimental soil, from 1990 to 2000, as soil potassium content increased so boron content declined, and as a result yields of the experimental crop of plantain declined. This could be associated with the repeated application of potassium fertilizer to the soil.

**Recommendation**

Further investigations are needed to improve accuracy of the recommendation for fertilization with boron in systems of plantain cultivation, in relation to boron content of leaves and based on different critical levels of extraction of the borate anion.

**Acknowledgements**

The authors would like to thank the Comité de Cafeteros del Quindío, for the economic support for the soil analyses, which were essential to the present study; Dr Fabio Aranzazu H., research scientist at CORPOICA, Regional 9; and Huberto Morales Osorno and Luz Dary Celis García, research assistants, CORPOICA, Regional 9.

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Recommendation

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**References**


Evaluation of the agronomic characteristics of plantain hybrids (Musa spp.)

P. Orellana Pérez, I. Bermúdez Caraballos, L. García Rodríguez and N. Veitia Rodríguez

Plantains are an important food source for the populations of Latin America and certain African countries. Plantains of the ‘Horn’ type, traditionally the most commonly grown, are seriously affected by black leaf streak (Mycosphaerella fijiensis Morelet), which has greatly reduced the supply of this product both in local markets and for export. This is the main disease which threatens the production of this source of both food and income (Jacome 1998).

The fact that plantains are usually grown on smallholdings, sometimes in mountainous areas and very often interplanted with other crops, makes chemical control of the disease difficult. The consequence, not only on volume of production but also on the quality of the product, is that current levels of production fail to meet the growing demand of certain local and export markets.

In the 1990s, the first plantain hybrids resistant to black leaf streak and destined for commercial use, developed by the Fundación Hondureña de Investigación Agrícola (FHIA), offered the possibility of introducing new clones into commercial production and of restoring adequate production levels at lower cost.

However, because of their genetic constitution, which includes a contribution from clones of the tall ‘French’ type, the new hybrids must be characterized morphologically and studied agronomically before being exploited commercially.

The work presented here shows the results of evaluation of various agronomic characters of the FHIA hybrids in the central region of Cuba.

Materials and methods
The studies were made on tissue-cultured plants produced by micropropagation using the method proposed by Orellana (1994), planted out on the multi-crop farm “La Cuba”, situated in the province of Ciego de Avila.

Fifty plants of each hybrid were planted 3 metres apart, in two furrows also situated 3 m apart. On 20 plants of each hybrid, starting at the beginning of flowering, the following characters were recorded:

- Plant height
- Number of functional leaves (with more than 75% of green leaf area) at the start of flowering (NFLF)
- Number of leaves showing lesions typical of black leaf streak (Stage 5 on the Stover scale modified by Gaulh (1984)) at the start of flowering (NL5H)
- Number of functional leaves (with more than 75% of green leaf area) at harvest (NFLH)
- Number of leaves with black leaf streak lesions at harvest (NL5H)
- Number of hands per bunch
- Length and diameter of the central finger of the first and penultimate hands
- Number of days between harvest and ripening (second hand at ripening degree 1, using the scale in ‘Descriptors for banana’ [(IPGRI-INIBAP/CIRAD 1996)])
- Growing period in days from planting to flowering and until harvest.

Using the results of counts of the number of functional leaves showing typical black leaf streak lesions, two formulae were devised to serve as indicators of the reduction in functional leaf area: the Functional Leaf Reduction Index (FLRI) and Relative Infection Index (RII), reflecting the damage caused by the disease. The latter index depends on the number of functional leaves showing typical lesions at the start of flowering and when the bunch is harvested.

Formulae:
- FLRI = NFLF/NFLH
- RII = FLRI X NLSH/NFLH = NFLF X NLSH/(NFLH)²

As there is only one production cycle in Cuban plantains, the counts all apply to the mother plant.

Results and discussion
The results show that with the exception of FHIA-19, which has the lightest bunches, the hybrids did not differ in their bunch weight. For all the hybrids, the majority of the weight of the bunch is concentrated in the first four hands (59.71% of the total weight). FHIA-19 had the highest proportion in these hands (71%), which is confirmed by the observation of the length and breadth of the fingers of the first hand (Table 1). The concentration of the majority of the weight in the first hands is a characteristic of the plantain. It justifies the removal of the terminal hands of hybrids that have developed more than eight hands per bunch in order to encourage greater development of the fingers in terms of length and diameter. This latter aspect is very important if the hybrids are to claim to rival the ‘Horn’ type plantains. No differences were observed between the hybrids as regards other bunch characteristics. Arcila et al. (2000) recommended leaving five hands and removing the rest 20 days after the start of flowering.

It is important to emphasize that the hybrids with the longest interval between harvest and ripening under natural conditions (31 days) are FHIA-20 and FHIA-22; for FHIA-21 it is only 8 days. This shows that the first two have advantages for local sale and for export over short distances.

As for response to black leaf streak, the FLRI shows that FHIA-04, which has only 1.3 functional leaves at harvest (FLRI = 9.31) is the hybrid whose leaf area was most reduced during the filling process of the fingers, resulting in insufficient filling of these fingers. The other hybrids had lower values of this index and similar for them all (Table 2). For these hybrids, the number of functional leaves at harvest was not less than four, allowing filling of the fingers.

The results indicate that hybrid FHIA-04 is also the most affected by black leaf streak, with an RII of 9.31 due to the fact that all its functional leaves bore typical lesions of the disease, which developed rapidly after flowering. At the time of harvest, FHIA-20 and FHIA-22, with more than two functional leaves unaffected by the pathogen, had the lowest values of RII: 1.38 and 1.40 respectively. FHIA-05, FHIA-19 and FHIA-21, although having higher values, responded well to the disease itself even though all their functional leaves at the time of harvest bore typical lesions. The results confirm that the time needed for the development of the disease on FHIA-04 was very much less than on the other hybrids, as already reported by Jones (1994).

The results suggest the possibility of using FLRI as an expression of the reduction of leaf area during the process of fill-
ing of the fingers, and RII as an expression of the time needed for the development of the disease as a function of the leaf area affected, given by the number of functional leaves and that of leaves which are necrotic at harvest time, a relationship which has always been difficult to quantify numerically.

According to Ortiz and Vuylsteke (1994), cited by Craenen (1998), it requires at least eight functional leaves during the whole growth period and a similar number of healthy leaves before flowering to guarantee a good yield.

From this point of view, FHIA-20 has the shortest growth period from planting to harvest, at 481 days, whereas for the other hybrids this period varies from 493 to 518 days.

Conclusions
• The hybrids FHIA-20 and FHIA-22 should have good yield potential owing to the longer period of time between harvest and fruit ripening. FHIA-20 also has the shortest growth period.
• The hybrids FHIA-05, FHIA-19 and FH-21 also have good yield potential. However, the RII shows us that by the time of harvest all their functional leaves are affected by black leaf streak, which in extreme infection conditions can have repercussions on yield.
• The indicators FLRI (functional leaf reduction index) and RII (relative infection index) proposed in this study, seem to be suitable for comparing the reduction in active leaf area and the time needed for black leaf streak to develop in different plantain clones during the period between flowering and harvest.

Table 1. Bunch characteristics and yields of the hybrids studied.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Bunch weight (kg)</th>
<th>Weight of first four hands (kg)</th>
<th>% of total bunch weight</th>
<th>Number of hands per bunch</th>
<th>Finger length (mm) First</th>
<th>Penult.</th>
<th>Finger diameter (mm) First</th>
<th>Penult.</th>
<th>Number of days from harvest to ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA-04</td>
<td>20.3 a</td>
<td>12.8</td>
<td>63</td>
<td>8.5</td>
<td>21.4</td>
<td>14.8</td>
<td>39.3</td>
<td>32.4</td>
<td>8</td>
</tr>
<tr>
<td>FHIA-05</td>
<td>21.5 a</td>
<td>13.5</td>
<td>63</td>
<td>8.6</td>
<td>20.8</td>
<td>15.5</td>
<td>39.0</td>
<td>33.5</td>
<td>8</td>
</tr>
<tr>
<td>FHIA-19</td>
<td>16.8 b</td>
<td>12.0</td>
<td>71</td>
<td>8.0</td>
<td>22.0</td>
<td>13.8</td>
<td>40.2</td>
<td>30.0</td>
<td>9</td>
</tr>
<tr>
<td>FHIA-20</td>
<td>20.6 a</td>
<td>12.1</td>
<td>59</td>
<td>9.7</td>
<td>19.0</td>
<td>14.0</td>
<td>39.8</td>
<td>32.0</td>
<td>11</td>
</tr>
<tr>
<td>FHIA-21</td>
<td>21.3 a</td>
<td>13.1</td>
<td>62</td>
<td>8.7</td>
<td>21.1</td>
<td>14.8</td>
<td>38.7</td>
<td>31.5</td>
<td>7</td>
</tr>
<tr>
<td>FHIA-22</td>
<td>22.2 a</td>
<td>14.0</td>
<td>61</td>
<td>8.6</td>
<td>20.0</td>
<td>14.0</td>
<td>41.0</td>
<td>31.0</td>
<td>11</td>
</tr>
</tbody>
</table>

[a, b]: means of values followed by different letters are significantly different according to Duncan’s multiple range test (P < 0.05).

First: Central finger of the first hand; Penult.: Central finger of the penultimate hand.

Table 2. Response of hybrids to black leaf streak attack.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>At flowering</th>
<th>At harvest</th>
<th>Flare</th>
<th>NLF</th>
<th>NFLF</th>
<th>NFLH</th>
<th>NLSH</th>
<th>FLRI</th>
<th>RII</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA-04</td>
<td>12.1</td>
<td>3.13</td>
<td>1.1</td>
<td>1.3</td>
<td>9.31</td>
<td>3.48</td>
<td>1.56</td>
<td>2.55</td>
<td>1.56</td>
</tr>
<tr>
<td>FHIA-05</td>
<td>10.2</td>
<td>3.80</td>
<td>4.0</td>
<td>4.0</td>
<td>2.55</td>
<td>1.48</td>
<td>1.48</td>
<td>2.55</td>
<td>1.56</td>
</tr>
<tr>
<td>FHIA-19</td>
<td>9.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>2.55</td>
<td>1.48</td>
<td>1.48</td>
<td>2.55</td>
<td>1.56</td>
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<tr>
<td>FHIA-20</td>
<td>12.7</td>
<td>4.0</td>
<td>5.0</td>
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<td>2.77</td>
<td>2.27</td>
<td>1.38</td>
<td>2.25</td>
<td>1.38</td>
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<td>FHIA-21</td>
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<td>4.5</td>
<td>4.5</td>
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<td>2.56</td>
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<td>5.5</td>
<td>5.5</td>
<td>2.27</td>
<td>2.27</td>
<td>1.40</td>
<td>2.25</td>
<td>1.40</td>
</tr>
</tbody>
</table>

Notes: NLF: Number of Functioning Leaves at flowering; NFL: Number of Leaves with Streak at flowering; NFLF: Number of Functioning leaves at flowering; NFLH: Number of leaves with streak at harvest; FLRI: Functional Leaf Reduction Index; RII: Relative Infection Index.

Options for in vitro propagation of the banana hybrid cultivar FHIA-20

L. García Aguila, B. Pérez Mederos, Z. Sarria Hernández, and J. Clavero García

Currently, several Musa cultivars are propagated by direct organogenesis from axillary buds by in vitro culture (Vasil 1994). This technique is the basis of mass propagation of bananas and plantains with the aim, for many countries at the present time, of large-scale commercial distribution of completely disease-free plants (Afza et al. 1996).

Genotype is known to have an influence on the efficacy of in vitro propagation and so, when new varieties or hybrid...
Materials and methods
For the study, young plants with a mean height of 25.6 cm, grown in the greenhouse, were selected (Figure 1). The process of introduction into the laboratory, including manipulation of the plants, disinfection of the corms, culture media for initiation and multiplication, together with the culture conditions, were as described by Orellana (1994).

The plants were cultivated in chambers under natural light conditions at a temperature of 27 ± 2°C. In every case, the base of the apex or bud was placed downwards on the culture medium.

Influence of the size of the apex and the physical state of the culture medium during the initiation phase
This study was made in order to establish the conditions for manipulation and growth of the apices during the initiation phase. For this purpose, the following treatments were studied (Figure 2):

1. 0.5 cm² apex cultured in liquid medium (control)
2. 0.5 cm² apex cultured in a semi-solid medium
3. 1.0 cm² apex cut in halves and cultured in liquid medium
4. 1.0 cm² apex cut in halves and cultured in semi-solid medium

At the end of 20 days’ culture, the following variables were recorded:
- Percentage regeneration of apices
- Percentage infection of apices
- Percentage mortality of apices
- Number of buds per apex

There were 20 replicates and the statistical method used to compare percentages was the comparison of proportions - ANOVA. The analysis of the variable “number of buds per apex” was a simple analysis of variance and the comparison of means was made using Tukey’s test at P < 0.05%.

Effect of doses of 6-benzylaminopurine and type of manipulation on the growth of buds during the multiplication phase
With the aim of resolving problems encountered during the growth of buds in the multiplication phase, the effect of a dose of 2 mg.L⁻¹ of 6-benzylaminopurine (BAP) was studied, the rate of 4 mg.L⁻¹, proposed by Orellana (1994) serving as control. Each dose was combined with two manipulation protocols.

Protocol 1. The buds were separated, decapitated 0.5 cm from the top and cut in two.

Protocol 2. Buds which had not reached 1 cm length were left in groups of two or were not separated from the mother plant, and there was no decapitation. Buds of more than 1 cm were separated, decapitated at this height and cut into two when the pseudostem was composed of more than three leaves.

Four treatments were thus obtained:
1. Multiplication medium with 4 mg.L⁻¹ BAP combined with protocol 1 (control)
2. Multiplication medium with 4 mg.L⁻¹ BAP combined with protocol 2
3. Multiplication medium with 2 mg.L⁻¹ BAP combined with protocol 1
4. Multiplication medium with 2 mg.L⁻¹ BAP combined with protocol 2

The variables recorded were the number of buds per initial explant and the percentage of buds growing as rosettes. The counts were made after three subcultures carried out every 21 days (culture in growth chambers with natural light and a temperature of 27 ± 2°C).

Five explants were inoculated in 250 ml flasks containing 30 ml of semi-solid culture medium (2 mg.L⁻¹ of Gellan gum (Spectrum)). There were 10 replicates. The data were analyzed by multifactorial variance and the means compared with Tukey’s test. Results in percentages were analyzed as in the previous experiment.

Results and discussion
Influence of the size of the apex and the physical state of the culture medium during the initiation phase
The use during the initiation phase of apices of 1 cm², cut in halves and placed on a semi-solid culture medium, gave 85% regeneration after 20 days of in vitro culture. On each section of the apex axillary buds were noted, guaranteeing a larger number of future explants at the multiplication phase, significantly different from that obtained with the other treatments (Table 1).

The technique of decapitating the apical dome proved necessary to induce the formation of new buds from the axillary buds, normally inhibited by apical dominance (Ma and Shi (1972) and Swami et al. (1983) (cited by Afza et al. (1996)). Pérez et al. (1998) emphasized the importance of increasing the multiplication coefficient during in vitro propagation of plantains, because each increase of this indicator by one unit corresponds to a cost reduction of about 10%.

In this study, mortality occurred only in apices cut and grown in liquid medium, possibly because the cutting procedure produced pieces which were too small to be grown in a liquid medium (Table 1). According to Orellana (1998), there are differences in tissue growth depending on the physical state of the culture medium: it proceeds differently according to whether the medium is solid or liquid.

The incidence of infection during this phase did not differ significantly with the treatment. However, various authors mention the influence of the size of the initial explant on the incidence of infection and have noticed that the smaller the explant, and the closer it is located to the apical meristem, the more the populations of micro-organisms fall (García and Noa 1998, Leifert et al. 1994).

Effect of doses of 6-BAP and type of manipulation on the growth of buds in the multiplication phase
By reducing the dose of cytokinin in the multiplication medium, the differentiation of buds into plantlets could be stimulated whilst little by little the growth of...
rosettes disappeared (inasmuch as the three subcultures were made with the 2 mg.L\(^{-1}\) dose of BAP). The manipulations or cuts made during this phase, combined with the reduction in the dose of cytokinin encouraged the biological response of the plants and caused the appearance of a larger number of buds per initial explant when using protocol 2 (Table 2). These buds, once transferred onto rooting media, had no difficulty in continuing their growth and reached the height, girth, and number of leaves needed to allow their transfer to the acclimatization phase.

On the other hand, for the treatments receiving the 4 mg.L\(^{-1}\) dose of BAP, we continued to note the appearance of rosette growth for both protocols, although the highest percentage of this type of growth occurred with the normal protocol (i.e. separation, decapitation at 0.5 cm and cutting buds into two). It seems that this treatment accentuates the presence of this particular growth in the clone FHIA-20: in fact, the percentage of rosettes tended to diminish when protocol 2 was used (Table 2).

The development of cultures in vitro requires an adequate ratio between auxins and cytokinins in the culture medium. One must also consider the endogenous concentrations of these hormones in the different types of explants or species (Jiménez 1998). Certain species are cultivated without the addition of any external regulator, probably because there is enough endogenous hormone present.

**Conclusions**

The results obtained from this work make it possible to propagate the hybrid FHIA-20 in vitro with a distinct improvement in the efficiency of the process of propagation by organogenesis by virtue of an increase in the number of buds. During the initiation phase it is necessary to culture apices of 1 cm\(^2\), cut into two, on a semi-solid medium. In this way, 85% of them regenerate plants by the end of 20 days’ culture. During the multiplication phase, the cytokinin dose should be reduced to 2 mg.L\(^{-1}\) in the culture medium and the explants separated into well-defined buds which are not less than 1 cm tall (those which are should be kept as pairs or should remain on the mother plant). Buds of 1.5-3.0 cm having more than three leaves can be decapitated at a height of 1.0 cm and cut in half. This way rosette growth is reduced by 2% and on average 4.7 buds per explant are obtained in the multiplication phase.

**Table 1. Behaviour of apices during the initiation phase after 20 days’ in vitro culture.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Regeneration of apices (%)</th>
<th>Number of buds per apex</th>
<th>Infection (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>40.0 b</td>
<td>0.25 c</td>
<td>15 a</td>
<td>0.0 b</td>
</tr>
<tr>
<td>2</td>
<td>40.0 b</td>
<td>1.10 b</td>
<td>20 a</td>
<td>0.0 b</td>
</tr>
<tr>
<td>3</td>
<td>35.0 b</td>
<td>0.00 c</td>
<td>15 a</td>
<td>40.0 a</td>
</tr>
<tr>
<td>4</td>
<td>85.0 a</td>
<td>2.42 a</td>
<td>15 a</td>
<td>0.0 b</td>
</tr>
</tbody>
</table>

*Identical letters in the same column indicate that the results do not differ significantly at P < 0.05%.

**Table 2. Growth behaviour of buds during the multiplication phase.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of buds per explant</th>
<th>Buds with rosetted growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>1.24 b</td>
<td>44.0 a</td>
</tr>
<tr>
<td>2</td>
<td>2.10 b</td>
<td>24.0 b</td>
</tr>
<tr>
<td>3</td>
<td>2.20 b</td>
<td>6.00 c</td>
</tr>
<tr>
<td>4</td>
<td>4.70 a</td>
<td>2.00 c</td>
</tr>
</tbody>
</table>

*Identical letters in the same column indicate that the results do not differ significantly at P < 0.05%.

**References**


The production of bananas and plantains is widely distributed in tropical and sub-tropical regions. The area cultivated, estimated to be around 10 million hectares, gives an annual yield in the order of 88 million tonnes. This crop, whose fruit forms part of the diet of more than 400 million people, ranks in the world in the category of staple food products, after rice, wheat and milk (FAO 1999).

In view of the interest in banana growing, considerable research effort has been devoted to the improvement and control of its mass propagation by means of biotechnological techniques such as somatic embryogenesis, for which three protocols have been described using vegetative tissue such as fragments of the corm and leaf bases (Novak et al. 1989, Ganapathi et al. 1999), cultures of proliferating meristems (Dhed’a et al. 1991, Dhed’a 1992, Schoofs 1997, Schoofs et al. 1998), and immature male or female flowers (Escalant et al. 1994, Grapin et al. 1996).

The use of cell suspensions in somatic embryogenesis and the discovery of the factors involved in the metabolic synchronization of cell suspensions and its timing constitute two fundamental aspects either of the procedures for applying methods of temporary immersion for mass micropropagation of economically important plant material (Escalant et al. 1994, Gómez-Kosky et al. 2000), or of their use in genetic improvement programmes by the induction of mutations, the study of selections in vitro (by means of fungal toxins or plant extracts) and genetic transformation by particle bombardment. Despite all the research carried out internationally in various laboratories, it is still difficult to maintain cell suspensions effectively. The setting up of banana cell cultures free from bacterial contamination, changes due to oxidation or possible fungal attacks requires a lot of time, and their maintenance therefore proves to be difficult (Schoofs et al. 1999). The aim of the work presented here was to determine, by using sources of carbon and growth regulators, the optimum experimental conditions for the setup and multiplication of a cell suspension on the one hand, and for the regeneration of the somatic embryos on the other.

Materials and methods

Maintenance of cell suspensions and homogenization of cultures

The plant material used to initiate the cell suspensions consisted of immature male flowers of Musa AAA cv “Grande naine” which had been placed on M1 induction medium [Murashige & Skoog (1962) salts - MS, 1 mg/L biotin, ANA and AIA, 4 mg/L 2,4-D, 6 g/L agarose, 30 g/L saccharose, pH 5.71] proposed by Grapin et al. (1998) for forming calluses. The friable embryogenic tissue obtained was transferred into M2 cell suspension medium [MS salts, 100 mg/L glutamine and malt extract, 1 mg/L 2,4-D, 45 g/L saccharose, pH 5.3] until its establishment. This technique for somatic embryogenesis was originally developed by Escalant et al. (1994) and it is currently applied in the biotechnology laboratory of CORBANA on this same clone (Acuña and Sandoval 2000).

From this initial suspension, new cultures were started during the phase of maintenance in M2 medium onto a medium made up of 35 ml of fresh M2 medium and 13 ml of the previous M2 medium (in which the suspension had been maintained during the preceding cycle), a mixture into which were introduced 2 ml of cells made up to a total volume of 50 ml per erlenmeyer flask. These suspensions were subjected to four treatments: T0 = 45 g saccharose; T1 = 45 g saccharose + 100 mg/L myoinositol; T2 = 30 g saccharose + 100 mg/L myoinositol; T3 = 15 g saccharose + 100 mg/L myoinositol. There were 10 replicates (Figure 1).

Four subcultures were made which were each incubated for 14 days as proposed by Escalant et al. (1994). The number of cells and the percentage viability of the suspensions were recorded on the 1st, 7th, and 14th days of the culture using a haemocytometer. There were three replicates and 5 counts for each, making a total of 15 readings per treatment. Also, every 15 days, the increase in cell volume was measured by

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Tissue culture | Cell suspensions

**Multiplication rate and regeneration potential of somatic embryos from a cell suspension of banana (Musa AAA cv. “Grande naine”)**
the sedimentation method (SCV) proposed by Schoof (1997) and the packed cell volume (PCV) as used by Reinert and Yeoman (1982). Four extra replicates were also made for monitoring the pH (2 in the inoculated medium and 2 in the non-inoculated medium), the measurements being made at the beginning and end of each subculture.

In order to evaluate the effect of the growth regulators on the quality of the cell suspension in the M2 medium, the treatment was selected which showed the highest multiplication rate and cell viability during the first four subcultures of the maintenance phase. For this study, we added to the selected M2 medium: A1 = 0.5 mg/L 2,4-D, A2 = 1 mg/L 2,4-D and A3 = 2 mg/L 2,4-D. The material was handled the same way as in the maintenance phase of the cell suspensions (mentioned above). The cell morphology was also noted, as clusters or solid masses, and photographs were taken using both optical and electron microscopes.

Regeneration of somatic embryos
The viability of the process was evaluated by observing the embryos obtained on the culture medium of Schenk and Hildebrandt (1972), called modified M3 [10 mg/L biotin, 100 mg/L of glutamine and malt extract, 230 mg/L proline, 1 mg/L ANA, zeatin and 2-IP, 10 g/L lactose, 45 g/L saccharose and a pH of 5.3]. The M3 medium was put into Petri dishes and sterile filter papers were placed on the surface on which were inoculated aliquots of 1 ml of the cells of treatments corresponding to the different growth regulator concentrations. The type of material regenerated was evaluated by making three evaluations per Petri dish from the zones where the distribution of the suspension was most homogeneous. All the cultures were maintained under conditions of controlled temperature (27ºC), relative humidity (80%) and photoperiod (12 hours).

Statistical analysis
The results obtained for the phase of maintenance of cell suspensions and homogenization of cultures for variation in pH, cell volume, number of cells and percentage viability, were analyzed by a linear modelling scheme and subjected to analysis of variance using the SAS programme (1990). Results showing heterogeneity of variance were adjusted to homogeneity by using a square root transformation.

Results and discussion
Effect of different saccharose or saccharose + myoinositol concentrations on the maintenance of cell suspensions and the homogenization of the cultures
The results for the increase in numbers of cells, presented in Figure 2, indicate that the dose of 30 g of saccharose provides enough carbon for the suspension, as its behaviour differed little from that of the suspension maintained with 45 g of saccharose. In general, the addition of myoinositol (T1-T2) did not alter the behaviour of the cells and a tendency was noted to stabilization in subculture 4 (relation of T1 and T2 with T10).

There were no significant differences between the percentages of viability of the treatments with and without myoinositol (T1 and T0 respectively). No differences were noted between the observations at different times (7 or 14 days) nor any interactions between the subcultures and time of observation (P = 0.1574).

On the other hand, as for the behaviour of this same percentage viability for treatments with myoinositol associated with different saccharose concentrations (T1 and T2), it was found that the differences for the four subcultures depended on the treatment (P = 0.0040). This difference in behaviour of distinct cell lines of a single clone may be an intrinsic characteristic of the material (Schoofs et al. 1999), which would indicate the need to redouble efforts towards improving these methods.

Treatment T3 (15 g saccharose + myoinositol) was eliminated because it showed progressive diminution of 5.18, 4.20 and 2.06 ml in subcultures 1, 2 and 3 respectively. This low success rate of cell proliferation may be attributable to the low availability of sugars in the medium compared with the demand of the cells in phase G1 of the cell cycle or to the osmotic shock due to the medium. Whichever it is, it is well known that saccharose as a source of carbon is a stabilizer of culture media (Takeuchi and Komamine 1982, Vardi et al. 1982, Smith et al. 1984).

The differences in cell volume found between treatments T0, T1 and T2 (P = 0.02602) were much reduced in subcultures 1 and 2 (Figure 3a and b) but were accentuated in subcultures 3 and 4 (Figure 3c and d) for which the difference between treatments T0 and T1 can be attributed to the action of myoinositol. Subcultures 2, 3 and 4 of T1 (with myoinositol) produced a mean cell volume 0.67 ml greater (P = 0.0188) than

![Figure 1. General scheme of the protocol followed to study a cell suspension of banana (cultivar "Grande naine"). a) Experiment 1: M2 cell suspension medium; S = saccharose; M = myoinositol; T = treatment; No. = replications. b) Experiment 2: Different concentrations of 2,4-D. c) Evaluation of embryo formation.](image-url)
that reached in the T0 subculture homologues. These results agree with those from other research on banana and other crops: for example Cronauer and Krikorian (1983) and Aftab et al. (1999) confirm the stimulatory action of myoinositol on mitosis and morphogenesis of plant cells.

The difference between the four subcultures was on average 0.23 ml in favour of treatment 1. The treatment which responded best was that containing 45 g saccharose and 100 mg/L myoinositol. It was also noted that the cell volume of subculture 1 was 5.95 ml and that of subculture 4 was 7.59 ml, representing a mean increase of 0.65 ml for each. There is a positive correlation (Figure 3e) between the number of subcultures and the cell volume, since this latter increases with the number of subcultures, finally to stabilize at the fourth subculture.

After mixing, the 35 ml of fresh medium and the 13 ml of old medium had a pH of 4.74. During the 14 days of culture, the non-inoculated media remained between pH 4.1 and 4.2 and the inoculated media between 4.4 and 4.6 (unpublished results). In the cell suspensions, the pH varied with time, treatment and their interaction (P = 0.0001): similar behaviour was observed by Skirvin et al. (1986). These same authors suggested that acidification of the medium could be due to ionic exchange between the cell and the culture medium, leading to an optimum pH for the normal functioning of the cell wall.

**Response of the suspension to different concentrations of 2,4-D**

Analysis of the results obtained for the variables “number of cells” and “percentage viability” on media containing different concentrations of 2,4-D did not show any marked differences in their behaviour. Treatments A1, A2 and A3 of the four subcultures had mean numbers of cells of 7.9, 6.0 and 7.0 and percentage viabilities of 59, 62 and 59 respectively.

When one studies the cell volume obtained with varying concentrations of 2,4-D (Figure 4) it is seen that treatment A1 (1 mg/L of 2,4-D) is that which maintains the best cell suspension with a mean volume of 7.6 ml and a maximum of 8.8 ml in subculture 3. The 2 mg/L dose of 2,4-D was the best for standardizing the cell volume of several subcultures, which is a useful parameter for carrying out studies of the cell cycle or cell metabolism and other phenomena connected with synchronized cell populations.

The final results from subculture 4 measured by the PCV method show that all the treatments have progressively increased the cell volume without large fluctuations during the 14 days of incubation and that this volume doubled on the sixth day, when the cells begin a phase of active division (Figure 5). These results agree with those obtained by Bieberach (1995) for various Musa clones.

As to the use of 2,4-D and appropriate doses, the results given here supplement the information about the action of this growth regulator on the embryogenic process and the doses required by different plant species. Lazzeri et al. (1987) emphasize the importance of auxins in the regulation of the somatic embryogenesis of soyabean and show that there is better somatic embryo production when 2,4-D is used alone than in combination with acetil a-naphthalene.

The morphology of cells in suspension was observed by optical microscopy at magnifications of 20x and 40x. The preparations showed cell clusters and...
isolated cells (Figures 6a and 6b), which conforms with descriptions by Grapin (1996) who reported that in suspensions of ‘French Sombre’ clusters were seen which could reach 70 to 80% of the volume of the suspension very similar to what we observed in the course of this work. The clusters are formed by pre-embryogenic cells (Figure 6c) possessing partitions or cellular plates typical of the last stage of mitosis and of cells which are empty or in the course of differentiation.

The isolated cells are round, with a dense cytoplasm and a well-defined nucleus: one may regard them as proto-

Figure 3. Cell volumes in the multiplication media of cell suspensions of banana (Musa AAA cv. “Grande naine”): a: means of treatments in subculture 1 (n = 10); b: means of treatments in subculture 2 (n = 9); c: means of treatments in subculture 3 (n = 9); d: means of treatments in subculture (n = 6); e: means of treatments in subcultures 0, 1, 2 (n = 23); T0 = 45 g saccharose; T1 = 45 g saccharose + myoinositol; T2 = 30 g saccharose + myoinositol; T3 = 15 g saccharose + myoinositol.

Bars represent the standard errors.

Figure 4. Cell volumes in the multiplication media of banana (Musa AAA cv, “Grande naine”). Mean of treatments A1, A2 and A3 containing 2,4-D in subcultures 1, 2, 3 and 4. Bars represent the standard errors.
plasts, initial cells with a primary cell wall that is characteristic of undifferentiated cells and with an active cell cycle. These observations are shared both by Bieberach (1995) who noted the presence of cells with identical morphological characteristics in cell suspensions of the cultivars "Dominico", "Grande naine" and "Gros Michel" and also by Sannasgala (1989) who described pre-embryos made up of protein bodies and starch.

The characters described above are a factor indicating the embryogenic condition of the cell suspension (Williams and Maheswaren 1986). Certain isolated cells with elongated cytoplasm containing lacunae are non-viable cells in a suspension because they have already formed their secondary cell wall.

Under the scanning electron microscope, round cells were seen of 50-80µm diameter, with rough walls with irregular ornamentations surrounded by a polysaccharide mucus (Figures 7a and 7b).

Regeneration of somatic embryos
Mixtures of cell samples treated with growth regulators were inoculated and maintained for 55 days on the semi-solid M3 medium to develop embryos. By the end of 22 days their growth began to be apparent, without traces of oxidation. The presence was detected of small clusters of 1 cm² containing globular embryos in the shape of a heart or torpedo. The embryos were sorted with a view to later regeneration of the plants (Figure 8a). A total of 200 “torpedo-type” embryos were transferred into eight Petri dishes at the rate of 25 embryos per dish. At the end of 20 days there was 63% germination, and after 41 days the plants possessed normal morphological characteristics. Until then the percentage germination for somatic embryos of the genus Musa oscillated between 45% and 80% according to the genotype and culture medium (Bieberach 1995, Escalant et al. 1995, Côte et al. 1996, Schoof 1997, Grapin et al. 1998).

Figure 5. Increase in cell volume (PCV) of a suspension of banana (Musa AAA cv, “Grande naine”) with different growth regulator concentrations. a, b, c: means of treatments (n = 10) in subculture 4.

Figure 6. Photomicrograph of cells and clusters, a: viable isolated cells, b: pre-embryogenic cells, c: clusters, N = nucleus, T = cell plates, C = clusters, CD = differentiating cells, CI = individual cells, CP = pre-embryogenic cell.
Figure 8b shows the potential regeneration of somatic embryos from cell suspensions. Escalant et al. (1994) have obtained the highest germination percentages, achieved by using temporary immersion systems with other banana cultivars.

Conclusions
These experiments have enabled us to standardize a protocol for obtaining embryos of Musa AAA cv. “Grande naine” from cell suspensions and by using growth regulators. The initial cell suspension was maintained with 45 g saccharose + 100 mg/L myoinositol. An initial pH of 4.74 and four subcultures of 14 days each guaranteed a sufficient cell volume and embryos with a good percentage viability. The optimal dose of growth regulator for the efficacy of the process is 1 mg/L of 2,4-D applied as an exogenous hormone. The morphological observations reveal that the protocol has allowed the development of viable cells which are easily transformed into embryos. The germination of embryos has validated the entire method and the doses used.

Acknowledgements
The authors are pleased to thank the Biotechnology Unit of the National Banana Corporation (CORBANA) of Costa Rica for permitting the experimental part of the work, and the University of Costa Rica, where the photomicrographs shown in this article were taken.

Note: Extract from the Biology thesis of Sandra Liliana Lerma defended before the Faculty of Sciences, University of Tolima, April 2001. Ibague, Tolima, Colombia.

References

Figure 7. Scanning electron microscope photographs. a: viable cells, b: external surface of a cell.

Figure 8. Formation of embryos of banana Musa AAA cv. “Grande naine” on M3 medium. a: 55-day old embryo cluster; (•) indicates “torpedo-type” embryos. b: germination and growth of embryos on M3 medium.
Introduction and multiplication of improved bananas and plantains in Nicaragua and distribution to farmers

K. Dens, M. Vargas, G. Matton, S. Coessens, I. Van den Houwe and R. Swennen

Banana and plantain in Nicaragua

Unlike most other Central-American countries, the production of banana and plantain in Nicaragua is low (Table 1). The major production zones of bananas and plantains are located in the coastal area near the Pacific Ocean. In the Chinandega region (North-West) an estimated area of 2000 ha Cavendish (Musa cv. AAA) is grown for export, while in the Rivas region (South of Managua) 13 000 ha plantain are cultivated for local consumption. For many small- and medium-scale farmers in Rivas, plantain is the most important crop. Gros Michel (Musa cv. AAA), Bluggoe (Musa cv. ABB) and Silk (Musa cv. AAB) plantains are cultivated all over the country, mainly by small-scale farmers in backyards. In the higher regions of Central Nicaragua, with altitudes up to 1300 masl, bananas are grown in combination with coffee or cacao. Bananas and plantains are also important for the people at the Atlantic Coast. Pelipita (Musa cv. ABB) and Red (Musa cv. AAA) bananas are found in some regions of Nicaragua.

Plantain (Musa cv. AAB) is most preferred because it is an attractive cash crop. The most common landsraces are False horn plantain with an average of only 25 fingers. The price of plantain on the local market is much higher than the price of the other bananas (Gros Michel, Bluggoe, Silk) because of its much larger finger size and longer green life. Cavendish only enters the local market as rejections after being rejected on the export plantations. Its price is even lower than that of Gros Michel. Over the last five years prices of plantain have continued to increase, which reflects the high demand and insufficient supply of bananas and plantains caused by poor cultivation practices, drought and pests and diseases.

Diseases and pests are the main problems; black Sigatoka (Mourichon et al. 1997) and plant material contaminated by weevils (Gold and Messiaen 2000) are the major constraints affecting the small-scale plantain producer. Another important problem, especially in the Leon-Chinandega region, is the uneven distribution of annual rainfall. Without irrigation banana and plantain yields are reduced due to the long dry season.

Creating multiplying effects

The objective of the intervention is to contribute to food security and food quality of resource-poor farmers by giv-

Table 1. Production, export and consumption data of banana and plantain in five Central American countries.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Guatemala</td>
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<td>802 545</td>
<td>576 900</td>
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</tr>
<tr>
<td>Honduras</td>
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<td>702 578</td>
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<td>596 900</td>
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ing support to banana and plantain cultivation. Food insecurity is very high in Nicaragua and the number of undernourished people increased from 1.2 million in 1991 to 1.4 million in 1998 (FAO 2001). The project focuses on the Leon-Chinandega region (Figure 1), where the poorest farmers live and where bananas and plantains could be part of more diversified agriculture systems, now that the cotton monoculture has disappeared.

In 1996, the Universidad Nacional Autónoma de Nicaragua (UNAN), based at Leon, and the Flemish Office for Development Aid and Technical Assistance (VVOB) started their intervention with technical assistance from the Katholieke Universiteit Leuven (KULeuven). Improved germplasm came from the Fundación Hondureña de Investigación Agrícola (FHIA) and the International Institute of Tropical Agriculture (IITA) via the INIBAP Transit Centre (Diekmann and Putter 1996). The company Bananic supported this intervention by covering the operational costs of the laboratory and field activities. Tissue culture facilities were set up at UNAN to produce high-value banana and plantain varieties. Selected varieties are evaluated at UNAN’s test farm before distribution to small-scale farmers (Table 2). They are sorted in four main categories by local farmers according to their comparison with Bluggoe, the locally known False horn plantain, Gros Michel and Silk. At harvest, palatability tests are conducted with the farmers. Extension personnel of the project teach relevant cultivation and field multiplication techniques. Partnerships were built with about 20 national and international organizations operating in Nicaragua (Table 3), to accelerate the distribution of improved plants and technologies, and to obtain a maximum feedback from the farmers.

Achievements
The project started in mid-1996. Rooted plantlets were sent by KULeuven for weaning in the small nursery of the UNAN farm in Leon, located a few kilometres from Leon City centre. These plants were used for the first test plots at the University farm. The tissue culture laboratory of UNAN was built in 1997. Tissue culture techniques were transferred from KULeuven to UNAN that produced plantlets to extend the test fields at the University farm. In cooperation with the Centro de Enseñanza Técnica Agropecuaria (CETA), workshops were organized in six communities in Chinandega.

In 1998, five extension brochures in cartoon style were developed for distribution to participating farmers (Figure 2). At the test farm of UNAN, a field collection was established containing both the introduced and the locally grown varieties (40 in total), and 2 plots of 36 plants of each variety were evaluated (Table 2).

In 1999, two trained Nicaraguan technicians of the tissue culture laboratory produced 6500 plants. One hundred and forty new test plots were planted in the northwestern region of Nicaragua, mostly in Chinandega because of the cooperation with CETA and the larger agriculture activity of that region.

In 2000, 20,000 plants have been distributed to 370 new farmers, comprising farmers of the Leon region as well (Figure 1). Ten thousands plantlets were imported from KULeuven to accelerate the distribution of the new varieties. Oxfam-Belgium contracted the UNAN to distribute 25,000 plants of superior varieties to nearly 1000 families that were relocated after the hurricane Mitch in October 1998 and urgently needed new plant material. Therefore the shade house was extended to 700 m².

In 2001, a few test fields were also planted in the Rivas, central Nicaragua and Atlantic coast regions, where a selection of farmers received in vitro banana and plantain plants.

The number of plants produced and distributed by UNAN’s tissue culture laboratory increased from 2000 in 1998 to 15,000 in 2001. The number of farmers participating also increased considerably - from 40 at the start of the project in 1998, to a total of 820 having received
Table 2. Harvest characteristics of 23 varieties obtained from test fields during their first and second cycles (C1-2) and third and fourth cycles (C3-4).
Varieties are grouped according to consumers' preference.

<table>
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<th>Name</th>
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<tr>
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<td>1205</td>
<td>AAB</td>
<td>125</td>
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<tr>
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<td>1205</td>
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<tr>
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<td>AAB</td>
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<td>Dessert bananas</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosal (Silk)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pisang ceylan</td>
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<tr>
<td>Yangambi km5</td>
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<td>AAB</td>
<td>125</td>
<td>186.4</td>
<td>*8.5</td>
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<tr>
<td>TMX1378 (BITA 3)</td>
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<td>AAB</td>
<td>125</td>
<td>186.4</td>
<td>*8.5</td>
<td>10.8</td>
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<tr>
<td>Pisang mas</td>
<td>1205</td>
<td>AAB</td>
<td>125</td>
<td>186.4</td>
<td>*8.5</td>
<td>10.8</td>
</tr>
<tr>
<td>AA cv. Rose</td>
<td>1205</td>
<td>AAB</td>
<td>125</td>
<td>186.4</td>
<td>*8.5</td>
<td>10.8</td>
</tr>
<tr>
<td>Pisang lidi</td>
<td>1205</td>
<td>AAB</td>
<td>125</td>
<td>186.4</td>
<td>*8.5</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Table 3. National and international partners involved in the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>INIBAP</td>
<td>International Network for the improvement of Banana and Plantain</td>
</tr>
<tr>
<td>KULeuven</td>
<td>Katholieke Universiteit Leuven</td>
</tr>
<tr>
<td>UNAN</td>
<td>Universidad Nacional Autónoma de Nicaragua</td>
</tr>
<tr>
<td>AUSTAR</td>
<td>Foundation for community development</td>
</tr>
<tr>
<td>ATC</td>
<td>Asociación de Trabajadores del Campo</td>
</tr>
<tr>
<td>BLOQUE</td>
<td>Evangelistic Association for Education of Farmers</td>
</tr>
<tr>
<td>CIPRES</td>
<td>Centro de Investigación y Promoción para el Desarrollo Rural y Social</td>
</tr>
<tr>
<td>SGJ/RH</td>
<td>Association of Garimendia Jiron with Limited Responsibility</td>
</tr>
<tr>
<td>UNAG</td>
<td>Unión Nacional de Agricultores y Ganaderos</td>
</tr>
<tr>
<td>UNAPA</td>
<td>Unión Nacional Agropecuaria de Productores Asociados</td>
</tr>
<tr>
<td>Xochilt Acat</td>
<td>Women Association of Malpaisillo</td>
</tr>
<tr>
<td>CETA</td>
<td>Centro de Enseñanza Técnica y Agropecuaria</td>
</tr>
<tr>
<td>INTA</td>
<td>Instituto Nicaragüense de Tecnología Agropecuaria</td>
</tr>
<tr>
<td>MAG-FOR</td>
<td>Ministerio de Agricultura, Ganadería y Forestales</td>
</tr>
<tr>
<td>CARE</td>
<td>North-American NGO</td>
</tr>
<tr>
<td>CATIE</td>
<td>Centro Agronómico Tropical de Investigación y Enseñanza</td>
</tr>
<tr>
<td>CUSA (USAID)</td>
<td>Cooperative League of USA</td>
</tr>
<tr>
<td>EU</td>
<td>European Union Project León-Chinandega</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>OXFAM-Solidarity</td>
<td>Oxford Committee for Famine Relief - Belgium</td>
</tr>
<tr>
<td>SI</td>
<td>Solidaridad Internacional - Spain</td>
</tr>
<tr>
<td>BANANIC</td>
<td>Corporación Bananera Nicaragüense</td>
</tr>
<tr>
<td>SETAGRO</td>
<td>Servicios Técnicos Agropecuarios de Occidente</td>
</tr>
</tbody>
</table>

improved varieties and participated in the project in 2001. During 2001, the planting material was sold to cooperating institutes who distributed the plants in their own development programmes.

A total of 2757 farmers were trained in different workshops and 1500 brochures dealing with field selection and preparation and plant material were distributed. Workshops for farmers were organized in collaboration with local NGOs, governmental and international organizations, which drastically increased the contact with farmers (Table 3). The project also participated in the organization of regional and national workshops for extension workers. Six new brochures were developed about diseases and pest control. A catalogue of the new accessions following the format of Musalogue (Daniells et al. 2001) was made available.

Close contact is being maintained with the farmers who are growing the new varieties (Figure 3) to assess their reaction and improve the efficiency of the intervention. Interviews are conducted to determine the acceptance rate of the new varieties and to identify the underlying reasons, e.g. appearance, taste, cultivation as a cash crop and/or food crop, etc. (Table 4). The most popular variety so far is FHIA-03, because of its drought resistance and large bunches that are comparable to the local Bluggoe cooking banana. Tasting sessions are organized on a regular basis and the new varieties are prepared according to prevailing Nicaraguan customs i.e. fried, green and ripe plantains, plantain chips, cooked green and ripe plantain, and banana as a dessert. People are asked to compare the new fruits with the local fruits (False horn plantain, Bluggoe or Silk). First results confirm the acceptability of most varieties but also show that palatability tests are absolutely necessary since varieties but also show that palatability

Table 5). The most popular variety so far is FHIA-03, because of its drought resistance and large bunches that are comparable to the local Bluggoe cooking banana. Tasting sessions are organized on a regular basis and the new varieties are prepared according to prevailing Nicaraguan customs i.e. fried, green and ripe plantains, plantain chips, cooked green and ripe plantain, and banana as a dessert. People are asked to compare the new fruits with the local fruits (False horn plantain, Bluggoe or Silk). First results confirm the acceptability of most varieties but also show that palatability tests are absolutely necessary since varieties but also show that palatability
Distribution and extension work will be increasingly coordinated by local organizations and NGOs. To facilitate this, the UNAN/VVOB staff participated in 2001 in the foundation of a national Musa network, MUSANIC.

A baseline study about the socio-economic situation of the collaborating farmers has been carried out to be able to measure the project-impact within a few years.

References


Table 4. Most preferred varieties and reasons for it (N = 80).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Most important reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>False horn Plantain* (Cuerno)</td>
<td>Market</td>
</tr>
<tr>
<td>FHIA-03</td>
<td>Drought-resistant, bunch size</td>
</tr>
<tr>
<td>TM Bx 5295</td>
<td>Good taste</td>
</tr>
<tr>
<td>Bluggoe* (Cuadrado)</td>
<td>Drought-resistant, firmness of fruit, taste</td>
</tr>
<tr>
<td>TM Bx 1378</td>
<td>Shape of fruit, taste</td>
</tr>
<tr>
<td>Pelipita</td>
<td>Firmness of fruit, taste</td>
</tr>
</tbody>
</table>

* local varieties.

Table 5. Acceptability of the fruit taste and visual aspect (N = 80).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mode of preparation</th>
<th>Compared with</th>
<th>% better or the same</th>
<th>Fruit taste</th>
<th>Compared with</th>
<th>% better or the same</th>
<th>Fruit visual aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA-03</td>
<td>Ripe</td>
<td>Bluggoe</td>
<td>95</td>
<td>Gros Michel</td>
<td>44</td>
<td></td>
<td></td>
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<tr>
<td>FHIA-01</td>
<td>Ripe</td>
<td>Gros Michel</td>
<td>62</td>
<td>Bluggoe</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pisang lidi</td>
<td>Ripe</td>
<td>Silk</td>
<td>37</td>
<td>Silk</td>
<td>13</td>
<td></td>
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<tr>
<td>TM Bx 1378</td>
<td>Ripe</td>
<td>Silk</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TM Bx 5295</td>
<td>Ripe fried</td>
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<td>86</td>
<td>False horn</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TM Bx 5295</td>
<td>Green fried</td>
<td>False horn</td>
<td>67</td>
<td>False horn</td>
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<td></td>
<td></td>
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<tr>
<td>TM Px 4479</td>
<td>Ripe fried</td>
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<td>False horn</td>
<td>49</td>
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<tr>
<td>TM Px 4479</td>
<td>Green fried</td>
<td>False horn</td>
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<td>Green fried</td>
<td>False horn</td>
<td>80</td>
<td>False horn</td>
<td>12</td>
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<tr>
<td>Pelipita</td>
<td>Ripe fried</td>
<td>False horn</td>
<td>37</td>
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<tr>
<td>Pisang ceylan</td>
<td>Ripe</td>
<td>Silk</td>
<td>72</td>
<td>Silk</td>
<td>91</td>
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</table>

Koen Dens, G. Matton and S. Coessens work as cooperants of VVOB at the UNAN; M. Vargas is Head of the Musa project of the UNAN, Laboratorio de Cultivo de Tejidos; Iglesia la Merced 1/2 C al N; Facultad de Ciencias, UNAN-León, Nicaragua; E-mail: vtro@unanleon.edu.ni; http://www.unanleon.edu.ni/~vtro/;

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Using RAPD technique for identifying and classifying some banana cultivars in Vietnam

Nguyen Xuan Thu, Le Thi Lan Oanh and Ho Huu Nhi

Banana is an important plant in tropical countries. Banana originates from Musa acuminata (AA) and Musa balbisiana (BB). Ten groups of cultivars with levels of ploidy ranging from diploid (2n = 2x = 22) to tetraploid (2n = 4x = 44) and different genomes are recognized. The following genomic configurations are known to exist: diploid AA, BB, and AB; triploid AAA, AAB, ABB; and tetraploid AAAAA, AABB, ABBB (Simmonds and Weatherup 1990). Therefore, a wide variety of classification and identification systems exists for banana. Up to now, the traditional classification and identification have been based only on morphology and quantitative traits. Recently, molecular markers have been used to study diversity on plants, animals and microorganisms.

The random amplified polymorphic DNA (RAPD) technique, which utilizes polymerase chain reaction (PCR) amplification with single primers of arbitrary nucleotide sequence, has been developed by Williams et al. (1990) and Welsh and McClelland (1990) to produce molecular markers for genetic analysis. RAPDs have been shown to be useful in genetic fingerprinting (Yang and Quiros 1993, Orozco-Castillo et al. 1994, Lanham et al. 1995). In this study, we have used RAPDs for identifying and classifying some banana cultivars.

Materials and methods

Materials

In this study, six indigenous banana cultivars of Vietnam (Table 1), which were obtained from the Institute of Agricultural Genetics (Vietnam) were screened for RAPD markers.

Isolation of DNA

DNA was isolated from banana leaves using the method of Murray and Thompson (1980) with some modifications. Four grams of fresh leaf material were ground in liquid nitrogen in presence of glass-sand. The powdered leaf tissue was stored at -20°C for two hours. Ten milliliters of extraction buffer (1.5% cetyltrimethylammonium bromide (CTAB); 100 mM Tris-HCl (pH 8); 20mM ethylenediaminetetraacetic acid (EDTA) (pH 8); 1.4 mM NaCl; 0.2% mercaptoethanol) heated to 65°C was added, and the mixture was then incubated at 65°C for 30 min. The mixture was shaken lightly with 1.5 volume of chloroform: isoamyl alcohol (24:1) for 20 min at room temperature. The sediment was removed by centrifugation at 3000 rpm for 20 min. The DNA was precipitated with the addition of 0.8 volume of freezing propanol (or 1.5 volume of 96% ethanol). The pellet was washed 2-3 times in 70% ethanol. At the end, DNA was redissolved in a minimum volume of TE (about 200 ml).

DNA amplification

Twelve primers from Operon Technologies, each ten bases in length, were used to amplify the DNA (Table 2). PCR was carried out in 25 ml reactions containing 20 ng of template (genome DNA), 200 mM of each dNTP, 2.5 units of Taq-polymerase, 15 ng of primers, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5mM MgCl₂, 0.001% (w/v) gelatine and 20 ml of mineral oil overlay. Forty-five amplification cycles were performed, each consisting of 94°C for 30 s, 36°C for 1 min, 72°C for 2 min. Products were analyzed by electrophoresis in 1.1% agarose gels at 100 V for 3 h, stained with 0.01% ethidium bromide and photographed under UV light.

Data analysis

• Coefficients of similarity among cultivars were calculated by using the formula of Nei and Li (1979):

\[ S_{ij} = \frac{2N_{ij}}{N_i + N_j} \]

where:

- \(N_{ij}\) = number of bands in common between cultivars \(i\) and \(j\), and
- \(N_i\) and \(N_j\) = number of bands for cultivars \(i\) and \(j\), respectively.

• The dendogram of cultivars studied was produced using the NTsyspc 2.0 computer programme.

Results and discussion

RAPD-PCR

Twelve primers were used to amplify the banana genome DNA. Nine of them amplified to give multiple PCR amplification products (Figure 1: example with primer H08), and three primers (G6, Y14, Y15) did not.

There were two kinds of bands: monomorphic bands which were present in all cultivars and polymorphic bands which were asynchronously present or absent in all cultivars. Nine primers were amplified into 79 bands, of which 67 bands (84.81%) were polymorphic and 12 bands (15.19%) monomorphic. The high proportion of polymorphic bands was due to the very different origin of the cultivars studied. Two primers (D07, G14) amplified 5 bands only, while H07 amplified 17 bands. The size of the bands ranged from 360 Kb to 3200 Kb.

Genetic similarity

The Nei and Li formula allowed to calculate coefficients of similarity among cultivars based on RAPD data. Similarity coefficients reflected the relationship between cultivars. Similarity coefficients between the original cultivars from M. acuminata ranged between 0.764-0.826, while those between the original cultivars from M. balbisiana ranged between 0.696-0.835 (Table 3). Cultivars belonging to the two groups had low similarity coefficients, ranging between 0.317 and 0.461.

Specific RAPD markers of cultivars

Specific RAPD markers are bands that are present only in one cultivar. In this study, we found 12 specific markers for 4 cultivars (Table 4). These results suggest that RAPDs can be used for the selection of banana breeds in agriculture.

Table 1. Cultivars and genotypes employed in the study.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Genotype</th>
<th>Cultivar</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chuoi Tieu Xanh</td>
<td>AAA (2n = 3x = 33)</td>
<td>4</td>
<td>Chuoi Tay</td>
</tr>
<tr>
<td>Chuoi Tieu Hong</td>
<td>AAA (2n = 3x = 33)</td>
<td>5</td>
<td>Chuoi La</td>
</tr>
<tr>
<td>Chuoi Mgu</td>
<td>AA (2n = 2x = 22)</td>
<td>6</td>
<td>Chuoi Hot</td>
</tr>
</tbody>
</table>
Phylogenetic tree of banana cultivars

Based on RAPD data, a phylogenetic tree of banana cultivars was constructed using the NTsyspc 2.0 program. The phylogenetic tree had two branches: the cultivars originating from *M. acuminata* were on one branch, and the cultivars originating from *M. balbisiana* were on the other branch (Figure 2). These results are in agreement with the cytological analysis of these banana cultivars.

Acknowledgements

The authors thank the Program of Fundamental Researches for supporting this research, and Inge Van den Bergh for reviewing this article.

References


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Consumption and expenditure patterns of banana and plantain consumers in Nsukka Urban, Nigeria

A.R. Ajayi and M.O. Aneke

In Nigeria, banana and plantain have always been very important traditional staple foods for both rural and urban population. They serve as a source of revenue for smallholders who produce them at the compound farms, mixed-cropping farms and small-scale sole cropping farms (Baiyeri 1996, Ajayi and Baiyeri 1999).

Nsukka Urban is heavily populated. It has a large market centre, operated on a daily basis. Men, women and the youths within Nsukka Urban and neighbouring communities converge at the market centre to buy and sell. Agricultural products such as banana, plantain, vegetables, pepper, mangoes, palm oil, other fruits, honey, yam, livestock, etc., are sold. In the area, banana and plantain are found in compound farms and they are mixed with other crops. Each of the banana and/or plantain growers in the area has less than 50 stands and a greater proportion of them grow more banana than plantain (Baiyeri and Ajayi 2000). However, banana and plantain marketing is most prominent among women, especially within Nsukka Urban, University campus and the neighbouring communities. The sale of banana and plantain provides means of livelihood for many households in the area.

In view of the significant contributions of banana and plantain to the economic, health and nutritional well-being of both rural and urban households in Nigeria, it is very important that efforts should be made continually to improved their marketing and consumption patterns. In planning such a national banana and plantain marketing and consumption improvement programme, data based on the consumption and expenditure patterns of their consumers in both rural and urban areas would be necessary. The role of Agricultural Extension (AE) in the gathering of data, planning, implementation, monitoring and evaluation of such a programme cannot be overemphasized. AE is a primary process through which the households can learn the reasons for change, the value of change, the results that can be achieved, the process through which change is achieved, and the uncertainties inherent in change (Williams 1978).

Expenditure patterns of households in Nigeria vary from place to place. Apart from the income of the households, factors such as preference of a particular product by a member of the household, quality and quantity of the product sold, environment under which the product was processed and sold, and the relative price of the products, also influence the expenditure patterns of the households (Anyanwu 1985).

Food consumption pattern, in a broad sense, means not only what the people eat or consume, but also the quantities as well as the forms in which these foods are consumed (Dury et al. 1999). According to Ologoke (1989), food consumption patterns vary from one place to another due to factors such as household size, educational levels of members of the household, relative prices of the food items, environment in which the consumers are living, social values attached to some food items, nutritive values of the food items, type or status of job performed by members of the household, household’s tastes and preferences, season/period of the year, and culture/religion of the household members.

The study was designed to assess the consumption and expenditure patterns of banana and plantain consumers in Nsukka Urban in Enugu State, Nigeria. Specifically, the study was designed to:
1. determine the consumption patterns of bananas and plantains among household members in Nsukka Urban;
2. determine the expenditure patterns of banana and plantain consumers in Nsukka Urban;
3. determine the decision-making role of household members in banana and plantain consumption in Nsukka Urban;
4. determine the major problems militating against effective consumption of bananas and plantains in the study area; and
5. draw implications for extension programme on improved and efficient preservation, processing, marketing and consumption of bananas and plantains in the study area.

Methodology
Nsukka Urban lies within the centre of Nsukka Local Government Area, Enugu State, Nigeria. The land area of Nsukka Urban is about 45.38 km² (Oformata 1995). It is made up of the following different sections (clusters): the University of Nigeria campus, Onuiyi, Odenigbo, Government Reserved Area (GRA), Odenigwe, Ugwoye, Umuyo, Ngwuru, Owerre, Makashi and Isiakpu.

Out of the above-listed 11 clusters, five were selected through simple random sampling. From each of the five clusters, 12 households were selected, using clustering and simple random sampling techniques. In all, a total of 60 households were involved in the study, and the head of each of the households was interviewed.

A structured questionnaire schedule was developed and used in obtaining relevant information from the consumers of bananas and plantains. The data collected were analysed through the use of percentage distribution and bar charts.

Results of the survey
Consumption and expenditure patterns of banana and plantain among households in Nsukka Urban are presented in the following figures and tables:

Consumption rate of banana and plantain
Figure 1 shows that the consumption rate of bananas is higher than the consumption of plantains.

Sources of banana and plantain for consumption
Most of the consumers depend on the market for their banana and plantain supply. A very small proportion of consumers produce their fruits regularly (Figure 2).

Period of the day that bananas and plantains are mostly consumed
It clearly appears in Figure 3 that people prefer to eat plantain (consumed boiled, roasted or fried) in the morning and at
night, and banana as a ‘snack’ in the afternoon.

Common forms of plantain meal among households
Respondents preferred fried plantain for breakfast. For lunch, pounded and roasted plantains are the most eaten. For dinner, plantain accompanied with rice, beans or yams is preferred (Table 1).

Expenditure rate of banana and plantain consumers
It is to be noted that plantains are more expensive than bananas (N121 or N15 per plantain finger and N5 per banana finger). This could explain why the majority of consumers surveyed buy banana more regularly than plantain (Figure 4).

Table 1. Forms of plantain meal among the households.

<table>
<thead>
<tr>
<th>Form of meal</th>
<th>Breakfast (%)</th>
<th>Lunch (%)</th>
<th>Dinner (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fried plantain + pap</td>
<td>28.5</td>
<td>4.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Pottage plantain</td>
<td>19.2</td>
<td>3.7</td>
<td>11.0</td>
</tr>
<tr>
<td>Plantain + beans</td>
<td>3.5</td>
<td>10.6</td>
<td>18.9</td>
</tr>
<tr>
<td>Plantain + rice</td>
<td>2.3</td>
<td>9.6</td>
<td>21.1</td>
</tr>
<tr>
<td>Boiled plantain + stew</td>
<td>15.1</td>
<td>5.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Pounded plantain + soup</td>
<td>1.2</td>
<td>25.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Plantain with yam (pounded)</td>
<td>4.7</td>
<td>10.6</td>
<td>17.8</td>
</tr>
<tr>
<td>Roasted plantain</td>
<td>3.5</td>
<td>24.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Boiled plantain</td>
<td>22.0</td>
<td>6.4</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* Multiple responses

Proportion of monthly income spent on banana and plantain consumption
Most of the respondents (Figure 5) spend only 1% of their income on bananas and plantains. The main factors that determine the percentage of their monthly income spent on the purchase of bananas and plantains are the availability of money, closely followed by the family interest, and then the price of the fruits (Figure 6 – NB: more than one factor was given).

Forms in which bananas and plantains are purchased in the market
Bananas are mostly purchased ripe, whereas the respondents prefer to buy unripe plantains (Figure 7).

Response of households to changes in banana and plantain prices
Table 2 shows that most of the respondents do not change their habits in buying bananas when the price increases, but they buy more if the price decreases. In the case of plantain, more than half of the respondents would buy

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1 N (Nigerian Naira) = 0.085 USD, March 2002.
fewer plantains in case of increased price and 75% would buy more in case of decreased price.

**Household decision-making role in banana and plantain consumption processes**

According to Table 3, wives play the most important decision-making role in banana and plantain purchasing and consumption process. On the other hand, the husband plays the major role in the utilization of the peels, while children play the greatest role in determining the purchasing interval and storage period.

**Major problems militating against effective consumption of bananas and plantains**

Three major problems militating against the effective consumption of banana and plantain fruits were identified by the respondents (Figure 8). These included storage problems such as pest (house rats and insects) attacks, over-ripening and mould formation due to sustained bruises; processing problems such as lack

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**Table 2.** Percentage distribution of respondents according to their response to change in banana and plantain price.

<table>
<thead>
<tr>
<th>Response to change in price</th>
<th>10% increase in price</th>
<th>10% decrease in price</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Banana (%)</td>
<td>Plantain (%)</td>
</tr>
<tr>
<td>Buy same quantity</td>
<td>75.0</td>
<td>41.7</td>
</tr>
<tr>
<td>Buy more quantity</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Buy less quantity</td>
<td>25.0</td>
<td>58.3</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Table 3.** Household decision-making role in banana and plantain consumption processes.

<table>
<thead>
<tr>
<th>Decision-making role</th>
<th>H (%)</th>
<th>W (%)</th>
<th>C (%)</th>
<th>H (%)</th>
<th>W (%)</th>
<th>C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of monthly income</td>
<td>43.3</td>
<td>56.7</td>
<td>0.0</td>
<td>30.0</td>
<td>70.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Quantity purchased</td>
<td>21.0</td>
<td>70.7</td>
<td>8.3</td>
<td>35.0</td>
<td>50.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Form in which fruits are purchased</td>
<td>8.3</td>
<td>75.0</td>
<td>16.7</td>
<td>30.0</td>
<td>60.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Processing</td>
<td>0.0</td>
<td>85.5</td>
<td>14.5</td>
<td>10.0</td>
<td>64.6</td>
<td>25.4</td>
</tr>
<tr>
<td>Storage</td>
<td>0.0</td>
<td>75.3</td>
<td>24.7</td>
<td>0.0</td>
<td>86.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Utilization of peels</td>
<td>50.0</td>
<td>11.7</td>
<td>38.3</td>
<td>70.0</td>
<td>4.7</td>
<td>25.3</td>
</tr>
<tr>
<td>Storage period</td>
<td>16.7</td>
<td>25.0</td>
<td>58.3</td>
<td>11.7</td>
<td>21.7</td>
<td>66.6</td>
</tr>
<tr>
<td>Purchasing interval</td>
<td>33.3</td>
<td>41.7</td>
<td>25.0</td>
<td>13.3</td>
<td>33.3</td>
<td>53.4</td>
</tr>
<tr>
<td>Quality purchased</td>
<td>18.0</td>
<td>82.0</td>
<td>0.0</td>
<td>27.0</td>
<td>73.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

H = Husband, W = Wife and C = Children
of technological know-how, unfavourable weather conditions, unavailability of processing mills and poor supply/lack of electricity, etc.; and transportation without any bruise. Chukwu (1996) observed that inadequate storage, insufficient distribution and lack of processing techniques result in large proportions of banana and plantain being transformed into wastage.

Conclusions
The analysis of these results leads to the following conclusions:
1. the consumption rate of bananas was higher than that of plantain;
2. a greater proportion of the respondents depended on the market for their supply of bananas and plantains;
3. bananas were consumed mostly in the afternoon, while plantains were mostly consumed in the morning;
4. plantain meals were prepared and consumed in various forms;
5. plantains were more expensive than bananas;
6. the major factors that determined the proportion (%) of the monthly income spent on bananas and plantains included availability of physical cash, family interest, price, quality and availability of fruits;
7. bananas were mostly purchased in a ripe form, while plantains were mostly purchased unripe;
8. households responded accordingly to change in banana and plantain prices;
9. wives played the greatest decision-making role in banana and plantain purchasing and consumption processes than their husbands and children; and
10. the major problems militating against effective consumption of bananas and plantains in the area included storage, processing and transportation problems.

Implications of the findings for extension programme on improved and efficient preservation, processing, marketing and consumption of bananas and plantains

1. Since fluctuation in the market price of bananas and plantains affected the consumption patterns of the households, there is a need for the establishment of a consumer cooperative organization for bulk purchase and retail sale of the fruits. Capable extension agents should be attached to each of the so formed organizations for the purpose of monitoring and evaluating the activities of members and giving relevant pieces of information if and when necessary.
2. To ensure effective preservation, processing and utilization of bananas and plantains, the State Agricultural Development Project (ADP) should organize workshops for the women marketers on methods of preservation, processing and efficient utilization of banana and plantain.
3. The fact that a greater proportion of the respondents purchased the needed bananas and plantains from the market centres and hawkers shows that there should be efficient distributing and marketing channels to enhance consumers’ access to the fruits as of when due. Thus, the Enugu State ADP should embark on efficient Musa postharvest handling, distributing and marketing strategy programme for the rural farmers and the sellers.
4. Even though the wives could be specially targeted (because of their largest decision-making role in banana and plantain consumption) by the Enugu State ADP to improve on the banana and plantain consumption and expenditure patterns among households through appropriate educational activities, the importance of the household as a working unit in extension practice should not be overlooked. Therefore, all members of the household should be intensively involved in any extension programme designed to improve consumption and expenditure patterns of banana and plantain in the study area.

References
Olagoke M.A. 1975. Food consumption patterns in the Obafemi Awolowo University. BA Research Project, Faculty of Agriculture, Ile-Ife, Osun State, Nigeria.

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Molecular characterization of dwarf banana plants (Musa spp.) using AFLP

Katholieke Universiteit Leuven, Faculty of Agricultural and Applied Biological Sciences, Laboratory of Tropical Crop Improvement, Leuven, Belgium

Dissertationes de Agricultura No. 515, 2002

Abstract

Banana plants are the most important world fruit crop and show a large diversity in shapes and sizes, of which one is the dwarf type. Unlike the normal type, the dwarf variant has a shorter pseudostem and wider leaves. Because these plants have the same number of leaves as the normal variant, their photosynthetic ability is not reduced and therefore the bunch size almost identically. In addition, its reduced height prevents it from toppling during tropical storms. These characteristics make the dwarf variant a valuable plant for the tropical banana farmer. Different naturally occurring dwarf varieties exist which have a normal variant, but this phenotype is often obtained by in vitro culture too, e.g. in an in vitro germplasm collection or during rapid in vitro multiplication.

Vos et al. (1995) described the amplified fragment length polymorphism (AFLP) technique as ‘a novel and very powerful DNA fingerprinting technique for DNAs of any origin or complexity’. This DNA fingerprinting technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA and involves three steps: (i) restriction of the DNA and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) gel analysis of the amplified fragments. With this method, sets of restriction fragments are visualized by PCR without knowledge of nucleotide sequence. The power of this DNA fingerprinting technique was assessed on a few world species, but not on Musa spp. Therefore, it was here first optimized for banana. In addition, the technique was adapted for non-radioactive detection of the AFLP patterns using a more recent detection method. Fluorescein labelled primers were used in the PCR reactions which allow the computer based separation and detection on a sequencing gel.

The assessment of the power of the AFLP technique and its variants three-endonuclease-(TE)-AFLP, cDNA-AFLP and the methylation sensitive amplified polymorphism (MSAP) technique for the characterization and the early detection of the dwarf type was performed in this study on a range of dwarf-normal banana pairs. The dwarf ‘Curare enano’ and its in vitro generated normal-sized off-type were first used for the optimization of the AFLP technique. Later, the analysis was extended to three naturally occurring dwarf varieties ‘Cachaco enano’, ‘Figue rose naine’ and ‘Prata ana’, which have a normal variant called ‘Cachaco’, ‘Figue rose’ and ‘Prata’, respectively. In addition, the extra dwarf ‘Dwarf parfitt’, the normal ‘Cavendish’ and the giant ‘Giant cavendish’ were analyzed.

Differential AFLP, TE-AFLP, cDNA-AFLP, cDNA-TE-AFLP and MSAP patterns were obtained and different levels of polymorphisms were observed between the dwarf and normal type depending on the technique, the primer combination and the variety. For each variety, a distinction could be made between the dwarf and the normal type. However, no dwarf specific fragment was found to be common for all dwarf varieties, which is an indication that (i) either the observed polymorphisms are not related to the phenotype, (ii) or the different varieties originated in a different way, (iii) or that several genes are involved in the process. Differential fragments were cloned and sequenced. Primers were designed on the obtained sequences and used with genomic DNA of the respective variety to confirm the differential and unique nature of the fragments. However, specificity was lost.

Besides the above-described analyses at the DNA level, some physiological assays were performed on the dwarf-normal banana pairs. The relation between dwarfism and gibberellic acid (GA) is described for several mutant dwarf species, e.g. rice and wheat, as well as some Musa spp. Two categories of dwarf types are described, i.e. a GA-(in)sensitive group and a GA-deficient group. The influence of GA was tested in this study on the in vitro growth of the dwarf-normal banana pairs. The (in)sensitivity appeared to be variety dependent, suggesting that the different dwarf varieties originated in a different manner and that other mechanisms may be involved than the GA pathway alone. When grown on ancymidol (a GA-synthesis inhibitor) in vitro growth of all the tested dwarf varieties was inhibited, which indicates that these plants are not GA-deficient, and the signal transduction after the synthesis must be impaired.

From these results we can conclude that the AFLP technique allows a fast and early fingerprinting of these particular dwarf banana types, that the mechanism behind dwarfism is complex and seems to involve gibberellic acid, (de)methylation... and that the dwarf varieties here analyzed probably originated via different mechanisms.

Reference


Iris Engelborghs
Diseases and pests are the major constraints to the productivity of bananas and plantains. Nematodes cause important yield losses in Latin America, West and East Africa and Asia. Usually, banana nematodes are controlled by nematicides. These are not only very expensive but also extremely toxic for non-target organisms, including the user, and they pollute the environment. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that biotrophically colonize the root cortex and develop an extramatrical mycelium which helps the plant to acquire water and mineral nutrients from the soil, in exchange for carbon as an energy source. In addition, AMF increase the ability of the plant to control the spread of soil-borne pathogens. In Musa the association occurs naturally when plants are transplanted into the field. The association of AMF with plant-parasitic nematodes and the beneficial effect of the mycorrhizal symbiosis on plant growth and nematode resistance/tolerance led to investigations into the potential of AMF to limit yield losses due to nematodes.

In the first part of our study, the relative mycorrhizal dependency (RMD) and the AMF-nematode interaction were studied in four Musa genotypes, selected for their known host plant response to nematodes (i.e. ‘Grande naine’, ‘Gros Michel’, ‘Pisang jari buaya’ and ‘Yangambi km5’). Mycorrhization with Glomus mosseae(AMF) resulted in a significant better plant growth, even in the presence of Radopholus similis and Pratylenchus coffeae. No differences in RMD were observed among the four genotypes. Glomus mosseae could protect ‘Grande naine’ and ‘Pisang jari buaya’ against R. similis and P. coffeae, since the nematode reproduction was suppressed. Only in the case of R. similis (Indonesian population with low pathogenicity) in ‘Pisang jari buaya’, no suppression was observed. However, when reproduction is already very low (due to low reproductive fitness of the nematode population and/or the resistant host plant response of the tested genotype), the presence of the AMF has no effect on the nematode reproduction. The AMF reduced the root necrosis, caused by P. coffeae. For R. similis, no reduction was observed. The nematodes reduced the frequency of mycorrhization, without reducing the intensity of the mycorrhizal association.

In the second part, the RMD and the AMF-nematode interaction were studied in Musa genotypes differing in root morphology. The influence of the AMF on the root system and the influence of the altered root system on the nematode reproduction were examined. Mycorrhization with G. mosseae resulted in a significant better plant growth, even in the presence of P. coffeae. The effect of AMF on the root system was related to the RMD of the genotype. Musa genotypes with a low RMD will not experience changes in the branching of their root system in response to mycorrhization. But in genotypes with a high RMD, the root system will be more branched. We showed that P. coffeae also affects the root system, by reducing the branching. The effect of AMF on the nematode reproduction was not very clear. The nematode population density tended to be reduced, but was not significant in the experiment with ‘Obino l’ewal’. In the root system, it appeared that the decreased branching caused by the nematodes was counterbalanced by the increased branching caused by the AMF. Therefore application of AMF could be used as a strategy to decrease susceptibility to nematodes.

In the third part of our study, AMF-nematode interactions were studied under in vitro conditions. Firstly, aseptic nematode cultures were established using alfalfa callus as a host tissue. Until now the lack of completely sterile culture systems limited in vitro nematode-host studies and AMF-nematode interaction studies. Secondly, three model systems were developed: Ri T-DNA transformed Daucus carota roots, in vitro Musa plants and in vitro Arabidopsis thaliana plants.

Finally, the transformed D. carota roots were used to study the AMF-nematode interaction under sterile conditions. The results reported in this study confirmed the suppressive effect of AMF on nematode reproduction. Glomus intraradices could suppress the R. similis, P. coffeae and to a lesser extent M. javanica population in the roots. The internal and external developments of the AMF were not affected by the presence of these plant-parasitic nematodes.

Although this in vitro system has several limitations, there are still many legitimate reasons to use this system to study the AMF-nematode interaction. The AMF develops appressoria, arbuscules and vesicles in the root cortex, produces profuse extraradical mycelium and spores and is completing its life cycle in vitro. The early colonization occurs in a similar way as under in vivo conditions. The nematodes, R. similis and P. coffeae, can infect and reproduce in the roots, and cause similar damage in the in vitro roots as in vivo roots. In addition, the effects of the interaction reflect those observed in vivo. Although the dixenic system used is artificial, it may represent a valuable tool for studying the AMF-nematode interaction, complementary to classical experimental approaches.
Farmer-participatory evaluation and dissemination of improved Musa germplasm

INIBAP is the executive agency of an important four-year project of the Common Fund for Commodities (CFC) which started in November 2001. The aim of this project is to contribute to improved food and income security for small-scale farmers in banana-based farming systems, through the distribution and evaluation of improved Musa hybrids suitable for local consumption and marketing. The project involves seven countries namely Democratic Republic of Congo, Ecuador, Guinea, Haiti, Honduras, Nicaragua, and Uganda.

The project will be implemented in two phases. The first phase includes the multiplication of planting material of at least ten improved Musa varieties in each country and the distribution of these plants to farmers for on-farm evaluation. At least 150 farmers per country participate in the trials. The second phase will consist of funding support in the form of loans to small-scale farmers to enable them to purchase planting material and essential inputs for the more wide scale production of improved hybrids. The project will also include market studies on the improved hybrids and the training of farmers in improved production techniques, focusing on the integrated management of pests and diseases. The main result from the project will be the increased production of improved Musa hybrids by small-scale farmers. These varieties will produce higher yields and will not require chemicals for pest and disease control. In addition, farmers and entrepreneurs will be assisted to set up banana-related businesses (the production of planting material for sale etc.), thus contributing to increased income generation for rural communities. The major beneficiaries will be small-scale banana farmers.

For more information on the project, please contact Suzanne Sharrock, project coordinator, at INIBAP Headquarters.

Africa

The 3rd International bacterial wilt symposium took place in South Africa - 4-8 February 2002

The 3rd International bacterial wilt symposium was attended by 110 scientists from all over the world who presented more than 100 papers either orally or as posters. Aspects discussed were: epidemiology, disease management, breeding and deployment for disease resistance, host response and disease development, pathogen genetics, diversity and diagnosis.

Bacterial wilt caused byRalstonia solanacearum is reported to be one of the major constraints for many important crops such as potato, tomato, groundnut, banana, tobacco and ginger. In many cases, the disease causes very significant yield losses.

There is still a big gap in the research progress between developed and developing countries. In developed countries, scientists are generally more interested in the molecular aspects of the pathogen such as pathogen genetics, diversity and diagnosis. Except for diagnostics, other aspects under study are not directly related to control of the disease. Therefore, research being carried out on disease control by scientists in developing countries, where the disease is more serious and widespread, needs to be strengthened. Some work on breeding and deployment for disease resistance has been carried out and good results have been obtained for groundnut and potato. However, very little has so far been done for many other important crops such as banana and ginger.

Pathogen genetics

Significant progress has been made in studies of the genome of R. solanacearum. The pathogen has a 3,716,413 base pair (bp) chromosome and a 2,094,509 bp megaplasmid, which taken together encode over 5000 proteins. The chromosome harbours all essential genes, whereas the megaplasmid is involved in the biosynthesis of various amino acids, cofactors, and fitness to environments. There are about 200 candidate genes for pathogenicity distributed both in the chromosome and megaplasmid. This information is essential in understanding the biodiversity in R. solanacearum in relation to host specificity. Traditionally, R. solanacearum strains are grouped into five races, based on host range, and five biovars based on the oxidation on certain carbon sources.

A new classification scheme has been proposed based on the molecular analyses of R. solanacearum. Strains of R. solanacearum are classified into four phytophylotypes such as Phytophylotype I ‘Asia’ (include biovars 3 and 4, race 1, 4 and 5), phytophylotype II ‘America’ (biovar 1 and 2, race 1, 2, and 3), phytophylotype III ‘Africa’ (biovar 1, 2), and phytophylotype IV ‘Indonesia’ (biovar 1, 2, Pseudomonas syzygii, and blood disease bacterium – BDB). This shows that strains of Indonesia, including P. syzygii which causes Sumatra disease of clove, and the BDB on banana, are separated from other strains.

Detection

Various diagnostic kits have been developed, especially for quarantine purposes and for monitoring the pathogen in symptomatic and latently infected plant materials, surface water, soil, vegetable washings, and processing waste. Methods used are selective isolation and enrichment, bioassay, immunofluorescence, serology, and polymerase chain reaction (PCR). For use in developing countries, serology methods such as ELISA are more appropriate as they are cheaper.

The American Plant Pathology Society will publish the papers presented in the Symposium.

Further information about the symposium is available from Dr Supriadi, Research Institute for Spice and Medicinal Crops, Jalan Tentara Pelajar No. 3, Bogor 16111, Indonesia. Fax: (0251) 327010.

Asia and the Pacific

Musa acuminata in Northern Borneo

A preliminary report on the status of Musa acuminata in Northern Borneo has recently been prepared by Markku Häkkinen and Edmond De Langhe. This report is based on a survey of Musa in Sabah, Sarawak and Brunei carried out by the first author in August 2001. Although the main focus of the survey was the section Callimusa, a large number of photographs of Musa acuminata were also taken. With the expertise of Edmond de Langhe, a tentative taxonomic identification of the plants has been made.

The photographs showed that all the plants exhibited the basic characteristics of M. acuminata, with the typical top-like male bud, the horizontal-to-oblique inflorescence and bunch, and rather slender fingers. The flowers appeared to be white-to-creamy, but the details were not visible in the photos.

The specimens in question were found to fall into four main categories:

- M. acuminata ssp. microcarpa or truncata
- M. acuminata of uncertain status
- Edible AA diploids
- Unclassified accessions.
A microcarpa-truncata cluster?
The most important result of the Häkkinen visit to Northern Borneo, an area previously little explored for wild bananas, is the domination of a wild Musa acuminata population with a combination of characteristics typical of the subspecies microcarpa and truncata. The occurrence of yellow-green male bud types is the first time this character has been recorded for these subspecies. It is possible that the two supposed subspecies may actually form one large and compound population, and further studies on this material in a field genebank are required to confirm the status of the accessions in this group.

M. acuminata of uncertain status
A number of accessions were characterized by a moderately to strongly imbricate male bud and a trend for the bract colour to be pink/red/purple. Male buds with visibly imbricate bracts are characteristic for the subspecies siamea and burmannica, but are not expected for the acuminata's in Borneo or the Indonesian islands, with the exception of Java. Further studies are required to confirm if these are edible diploids or indeed truly wild plants.

Edible AA diploids
A number of plants are recorded as edible AA diploids. Amongst these are plants that were found to be populating large areas along the sides of roads, as truly wild populations do. Since there were no villages in the proximity, these may be the remnants of human population from remote time.

Conclusions
The findings presented in this report are clearly of a preliminary and tentative nature, as they are based on studies of photographs only. However, it does provide a basis for further studies, which the authors hope will be stimulated by this report.

Copy of the report, including colour photographs of all the accessions, are available in PDF format from the INIBAP website (http://www.inibap.org/publicationsborneo.pdf) or in printed form from INIBAP Headquarters, Montpellier.

Study of the association between nematodes and bananas in Vietnam
Inge Van den Bergh, a VWOB/INIBAP Visiting Associate Scientist, was based at the Agro-biotechnology Department of the Vietnam Agricultural Science Institute (VASI), Hanoi, Vietnam from October 1997 to December 2001 to carry out a study on the association between nematodes and bananas in Vietnam.

The project was funded by ACIAR (Australian Centre for International Agricultural Research), INIBAP and VVOB (Flemish Association for Development Cooperation and Technical Assistance).

The project had two main goals:
1. capacity building: to improve the local infrastructure for nematological research and to train local scientists in the field of nematology;
2. scientific research: to gain more insight into the different aspects of the association between nematodes and bananas in Vietnam, in order to improve local banana production. Specifically:
   - to obtain a more detailed picture of the occurrence of different nematode species on bananas in various regions of Vietnam;
   - to increase the knowledge about population-dynamics and the damage and yield loss potential of the most important nematode species on bananas;
   - to screen Vietnamese Musa germplasm for resistance to the most important nematode species.

Activities undertaken and results achieved

Capacity building
The general infrastructure of the laboratories was improved and various items of equipment were bought. An e-mail/internet connection was established in order to improve the communication between the different project partners and with other nematologists in the world, and to have access to information from the worldwide web.

During the time of the project, two staff members of the Agro-biotechnology Department of VASI followed the postgraduate international nematology course in Belgium. In this respect:

Scientific research carried out by the VWOB/INIBAP Visiting Associate Scientist
1. Assessment of the occurrence and distribution of nematodes on wild Musa species in natural habitats in North Vietnam
Three survey trips were undertaken in natural habitats in North Vietnam and root samples from three species of wild bananas were taken. With the exception of R. similis, the most important Musa nematode species, i.e. Meloidogyne spp., P. coffeeae and Helicotylenchus multicinctus, were found. This indicates that the natural soils in Vietnam are infested with these nematode species and that the three wild banana species are susceptible to these species.

2. Assessment of the occurrence and distribution of nematodes on Musa cultivars in North and Central Vietnam
Five survey trips were undertaken in six provinces in North and Central Vietnam and root samples of three commonly cultivated banana genotypes were taken. Again, Meloidogyne spp., P. coffeeae and H. multicinctus were found, but not R. similis. Damage parameters showed a clear relation with the presence of certain nematode species in the roots. For example, root-knot galling was positively correlated with the number of Meloidogyne spp. in the roots, while root necrosis was positively correlated with the number of P. coffeeae found in the roots.

3. Influence of a Pratylenchus coffeeae population on Meloidogyne spp. on plant growth and yield of banana
A field was planted with over 150 banana plants, of which one third was inoculated with P. coffeeae, one third with Meloidogyne spp. and one third was kept nematode-free (control plants). The preliminary results showed that infection with P. coffeeae and Meloidogyne spp. can reduce the plant height and the number of standing leaves in comparison with the uninfected control plants.

4. Population-dynamics of a Pratylenchus coffeeae population collected on Musa in North Vietnam
The reproduction of P. coffeeae on carrot discs under in vitro conditions could be described by the Gompertz equation: log (nem + 1) = 0.725 + 2.561 exp [-exp (1.742 (5.044 - time))].

From a greenhouse experiment that was repeated monthly over a period of one year, it could be seen that temperature has a strong effect on the reproduction rate of P. coffeeae on bananas. During the winter months, the reproduction was very low, while during the summer months, the population increased significantly. The extent of root-necrosis followed more or less the same pattern.
A greenhouse experiment was set up to assess the influence of irrigation on the reproduction of P. coffeae. A shortage of water had a very strong negative effect on the plant growth, while the nematodes could still reproduce well. A very high application of water reduced the general plant growth slightly, but the nematodes could barely reproduce. An intermediate water volume was best for the growth of the plants, but also favourable for the nematode reproduction.

The reproduction of a P. coffeae population on banana plants in the field was followed for more than one year. From preliminary results, it can be seen that temperature and rainfall have an effect on the reproduction rate of the nematodes.

5. Screening of Vietnamese Musa germplasm for resistance to a Pratylenchus coffeae population in the greenhouse

Twenty-four Vietnamese banana genotypes were screened for resistance to P. coffeae in the greenhouse. The most promising genotypes are 'Tieu xanh', 'Tieu xanh nam', 'Com chua', 'Com lua', 'Man' and 'Ngu tho'.

6. Screening of Vietnamese Musa germplasm for resistance to Meloidogyne spp. in the greenhouse

Twenty-two Vietnamese banana genotypes were screened for resistance to Meloidogyne spp. in the greenhouse. No sources of resistance were found.

7. Screening of Vietnamese Musa germplasm for resistance to Meloidogyne spp. in the field

Eight Vietnamese banana genotypes, 'FHIA-01', 'FHIA-02' and 'Yangambi km 5' were evaluated for their host plant response to Meloidogyne spp. under field conditions. 'FHIA-01', 'Ng-Thoc', 'Tay' and 'Com lua' were found to be less susceptible to Meloidogyne spp. 'FHIA-01', 'Ben tre' and 'Bom' were less sensitive to the knot-forming activity of Meloidogyne spp. The number of juveniles recovered from the roots was strongly influenced by the weather. During the cold and dry season, the numbers dropped very significantly. The number of egg-laying females in the roots (ELF) was much less influenced by the environmental conditions: there was a stagnation during the cold and dry season but no decline. Meloidogyne spp. seem to overwinter as eggs in egg-masses. Root-knot galling and ELF can be used as easy parameters to estimate the infection of Musa with Meloidogyne spp. No effects of the nematodes on plant growth were found. The number of nematodes in the roots seems to be related to the physiological stage of the plants. The highest nematode numbers were found during flowering.

Scientific research by Duong Thi Minh Nguyet and Nguyen Thi Tuyet

Duong Thi Minh Nguyet started a research programme on the occurrence of Radopholus similis in Vietnam and its morphological and biological aspects. Two surveys were carried out in Tay Nguyen (Western Highlands) to assess the occurrence of R. similis on coffee, durian, bananas, etc. One R. similis population was collected from durian roots and is being maintained on carrot discs under in vitro conditions. Since R. similis is still a quarantine pathogen in Vietnam, Duong Thi Minh Nguyet went to Belgium for a period of three months to study the collected population. She determined that the optimal temperature for reproduction of R. similis from Vietnam on carrot discs was 25°C. She also compared the reproduction of the Vietnamese population with populations from Indonesia and Uganda.

Nguyen Thi Tuyet is studying the biodiversity of Pratylenchus coffeae in Vietnam. She is collecting P. coffeae populations from various crops and places in Vietnam to study the morphological, biological and genetic diversity between the different populations.

Integrated management of Panama wilt disease of banana in Kerala

Banana wilt caused by Fusarium oxysporum f. sp. cubense is one of the serious threats to banana cultivation in Kerala. The acidic soils of the state and the susceptibility of the major commercial varieties offer an easy spread of the disease throughout the region, causing a yield loss of 10-15%.

The symptoms of the disease appear with the yellowing of older leaves which extends rapidly from the margin towards the midrib. These leaves hang withered around the pseudostem and the infection spreads to all leaves except the top, which hang down. The heart leaf also withers after 3-4 weeks. The plant exhibits longitudinal splitting with bulging and elongation of the pseudostem. When the rhizome is cut open, the discolouration of the vascular bundles can be seen and the cut stem smells of rotten fish.

A survey conducted by Estelitta et al. revealed that the disease was prevalent in all the districts in Kerala causing serious damage to the crop. It was also noticed that 'Nendran', the most important commercial variety in the State was susceptible to Panama wilt while Cavendish group, 'Palayankodan', 'Kapooravally' and cooking varieties such as 'Monthan', 'Kanchikela', etc. were not seen to be affected by the disease.

Several studies were conducted at the Banana Research Station, Kannara under Kerala Agricultural University on integrated management practices for Panama wilt disease. The pathogen was found to be soil borne and its entry to the host plant was through roots. Since the conidia can survive in the soil for as long as seven years, a package of agronomic practices are recommended based on the findings of the studies.

Field preparation should be carried out in a systematic way. In disease affected locations, weathering the pits for a week or more and burning the soil with dry leaves is recommended. Field sanitation, especially removal of grass weeds is necessary as they become critical alternate hosts.

Cultivation of tolerant varieties is suggested for disease prone areas. In other cases, suckers should be selected from disease free areas. Dipping pared suckers in 0.2% solution of carbendazim was also found to be an effective prophylactic measure.

It was also found that the application of lime at 1 kg per plant as a soil amendment at the onset of the monsoon and
good drainage were helpful in checking the disease.

The studies further indicated that use of organic manures in banana cultivation could give a better stand of the crop against the disease, probably due to improved soil structure with more aeration.

In the case of disease occurrence, removal and destruction of the diseased plants is recommended to check its further spread. Application of lime at 0.5-1 kg in the diseased plant pits and in the basins of surrounding plants also gave encouraging results in checking the further spread of the pathogen.

From experiments carried out on different chemicals to control Panama wilt, it was found that corm injections with 2% solution of carbendazim at 3ml/corm during the 5th, 7th and 9th month after planting could help to control the disease. Drenching the soil with 0.2% carbendazim was also found to be effective.

Since the commercial banana varieties in Kerala are often cultivated in extensive wetlands, studies indicated that crop rotation, intermittent fallowing or flooding followed by fallowing are also effective ways of reducing the spread of the disease.

Further information is available from S. Estellita, Associate Professor, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, S. India.

Cross compatibility of some banana clones

Before initiating a hybridization programme in banana breeding, cross-compatibility between desirable parents has to be assessed. Such work is presently going at Tamil Nadu Agricultural University (TNAU) in India. Seventeen varieties, including commercial triploids, diploids and TNAU-bred synthetic hybrids were included in the study (see Table 1). Anthers were collected from the male parents just prior to dehiscence and the pollen grains were extracted and smeared on the stigma of the female flowers of female parents on the day of opening in the early morning between 6.00 to 9.00 a.m., when the receptivity of stigma was good and ensured by stickiness by touch. After pollination, the flowers were covered with perforated paper bags. Once ripe, the fingers were longitudinally cut and the seeds, if present, were extracted carefully.

Among the 74 cross combinations tested, compatibility was found only in eight combinations (Table 2), thus indicating the existence of compatibility between clones. The successful crosses were between diploid x diploid and triploid x diploid. Out of the 10 male parents tested, 'Pisang lilin' and 'Anaikomban' were compatible with all female parents. 'Nendran' which was earlier reported as female sterile (Alexander M.P. 1970) was found to be a fertile clone. Seed production was very low despite the crosses being compatible.

Table 1. Details of parents used.

<table>
<thead>
<tr>
<th>Female parents</th>
<th>Male parents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triploids</strong></td>
<td></td>
</tr>
<tr>
<td>Karpooravalli (ABB)</td>
<td>Robusta (AAA)</td>
</tr>
<tr>
<td>Red banana (AAA)</td>
<td>Red Banana (AAA)</td>
</tr>
<tr>
<td>Rashthail (AB)</td>
<td></td>
</tr>
<tr>
<td>Nendran (AB)</td>
<td></td>
</tr>
<tr>
<td><strong>Diploids</strong></td>
<td></td>
</tr>
<tr>
<td>Nivediyakadalli (AA)</td>
<td>Nivediyakadalli (AA)</td>
</tr>
<tr>
<td>Mati (AA)</td>
<td>Pisang lilin (AA)</td>
</tr>
<tr>
<td>Sannachengadalli (AA)</td>
<td>Sannachengadalli (AA)</td>
</tr>
<tr>
<td>Anaikomban (AAA)</td>
<td>Anaikomban (AA)</td>
</tr>
<tr>
<td>Ambalakadalli (AA)</td>
<td>Ambalakadalli (AA)</td>
</tr>
<tr>
<td>Ney poovan (AB)</td>
<td>Erachvazhi (AA)</td>
</tr>
<tr>
<td><strong>Synthetic hybrids</strong></td>
<td></td>
</tr>
<tr>
<td>H-59 (AA)</td>
<td>H-59 (AA)</td>
</tr>
<tr>
<td>H-97 (AA)</td>
<td>H-97 (AA)</td>
</tr>
<tr>
<td>H-66 (AAA)</td>
<td>H-66 (AAA)</td>
</tr>
<tr>
<td>H-201 (AB)</td>
<td>H-201 (AB)</td>
</tr>
</tbody>
</table>

Table 2. Mean seed set in successful crosses.

<table>
<thead>
<tr>
<th>Name of cross</th>
<th>No. of flowers pollinated</th>
<th>No. of seeds obtained</th>
<th>Mean seeds / fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>3x x 3x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karpooravalli x Robusta</td>
<td>150</td>
<td>187</td>
<td>1.250</td>
</tr>
<tr>
<td>3x x 2x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karpooravalli x Pisang lilin</td>
<td>185</td>
<td>5</td>
<td>0.327</td>
</tr>
<tr>
<td>Karpooravalli x H-110</td>
<td>120</td>
<td>8</td>
<td>0.667</td>
</tr>
<tr>
<td>2x x 2x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matti x Pisang lilin</td>
<td>30</td>
<td>30</td>
<td>1.000</td>
</tr>
<tr>
<td>H-201 x Anaikomban</td>
<td>102</td>
<td>152</td>
<td>1.490</td>
</tr>
<tr>
<td>H-201 x Pisang lilin</td>
<td>79</td>
<td>427</td>
<td>5.405</td>
</tr>
<tr>
<td>H-201 x H-110</td>
<td>53</td>
<td>90</td>
<td>1.700</td>
</tr>
<tr>
<td>H-201 x Anaikomban</td>
<td>11</td>
<td>24</td>
<td>2.182</td>
</tr>
<tr>
<td>H-201 x Pisang lilin</td>
<td>12</td>
<td>4</td>
<td>0.333</td>
</tr>
<tr>
<td>H-201 x Robusta</td>
<td>11</td>
<td>1</td>
<td>0.091</td>
</tr>
</tbody>
</table>

References


Collecting banana germplasm in northeastern India

The northeastern states of India, namely Assam, Arunachal Pradesh, Meghalaya, Tripura, Mizoram and Manipur are a rich source of natural diversity in Musa. Since 1998, INIBAP has been supporting a series of Musa collecting missions in this region. These have been conducted by the National Research Centre for Banana (NRCB), Trichy. Specimens of the wild species Ensete glaucum, Musa balbisiana, M. acuminata, M. ornata and other Rhodochlamys species have been collected, together with a range of cultivated varieties. All the collected material has been established in the NRCB genebank for formal identification and characterization.

In Arunchal Pradesh, it was noted that where Eumusa and Rhodochlamys...
northeastern India.

Table 3. Details of wild Musa species collected during the exploration in northeastern India.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Section</th>
<th>Species</th>
<th>Site of collection</th>
<th>No. of accessions</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ensete</td>
<td>E. glaucum</td>
<td>Assam, Tripur, Mizoram</td>
<td>1</td>
<td>Fibre, vegetable, ornamental</td>
<td></td>
</tr>
<tr>
<td>Musa</td>
<td>M. balsiana</td>
<td>Assam, Tripur, Mizoram</td>
<td>1</td>
<td>Fruit, vegetable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. acuminata</td>
<td>Assam, Tripur, Mizoram</td>
<td>9</td>
<td>Fruit, vegetable</td>
<td></td>
</tr>
<tr>
<td>Rhodochlamys</td>
<td>M. ornata</td>
<td>Assam, Mizoram</td>
<td>1</td>
<td>Ornamental</td>
<td></td>
</tr>
</tbody>
</table>

Training course on MGIS in Africa

22–27 April 2002, CARBAP, Nyombé, Cameroon

A training course on the use of the Musa Germplasm Information System (MGIS) for the management of information related to genetic resources of bananas and plantains (Musa spp.) was held recently for Musa germplasm curators from Africa. The objective of this training course was to provide these curators with the expertise and tools to better manage information related to the accession in their collections. The use of MGIS will also allow them to exchange genetic resource information with other researchers and curators throughout the world. This training course was held thanks to funding support provided by the Technical Centre for Agricultural and Rural Cooperation (CTA).

The training course gathered 23 participants from West, Central, Eastern and Southern Africa (see list below). The course was held in French and English, with translation ensured by the trainers. All documents and training materials were provided in both languages.

The course included both field and computer-based training. Exercises on the taxonomical and botanical identification of varieties were held in the field using the list of descriptors published by CGI, INIBAP and CIRAD. The large germplasm collection maintained by CARBAP provided an excellent resource for the field exercises. This collection consists of over 400 accessions, representing a very large range of African varieties, especially plantains, but also including some East African highland varieties.

With regard to the computer-based training, participants learnt how to install the MGIS software on their computers, how to create users accounts, enter new records, and carry out information searches in the global database. They were also trained in the procedures for data exchange through the global database.

INIBAP News

New staff
Hélène Laurence, an intern funded by the Ministère des Relations internationales du Québec joined INIBAP as a Research Assistant in January 2002. She is based at the Regional Office of INIBAP in Kampala and will begin work on a 3-year study of the impact of improved banana varieties (Musa) on the livelihoods of households in Eastern Africa. She will work closely with INIBAP scientists and regional NARS partners. Hélène has a BSc in geography and a MSc in Agrometeorology.

Olivier Guinard, also funded by the Ministère des Relations internationales du Québec, joined the INIBAP programme in Montpellier as an intern in April 2002. During his 6-month internship he will be working on a project using the MGIS database (Musa Germplasm Information System) to carry out a series of tracer studies on important germplasm accessions as well as visualizing geographical assignments for the different accessions. Olivier comes from Québec where he studied for his BSc in Biology, specializing in molecular biology/biotechnology at the University of Québec in Montréal and has just completed his MSc.

Musaid.win, a software developed by CIRAD to assist in the identification of unknown varieties, was also explained by Xavier Perrier from the Biometry Unit of CIRAD. This software is provided to all participants in MGIS.

Participants agreed that the training provided a useful tool for the management of genetic resource information and highlighted the importance of collecting and managing this information using a standard format. The workshop also provided a valuable opportunity for curators to make contact with their colleagues from the region as a whole, and also to identify resource people who can help them in their future work.

Following the MGIS training course, a workshop on the “Names and synonyms of plantains” was held with the participants from West and Central Africa. Dr Kodjo Tomekpe conducted the workshop using data and photos from MGIS. A first draft list of variety names has been established that should be confirmed by further studies of the varieties in the field. INIBAP gratefully acknowledges the valuable support of CTA and CARBAP in the organization of the training course.

List of participants

From national organizations

Sylvestre M. Rogers, Rice Research Station, Rokupr, Sierra Leone;
Guilavogui Zeze, Institut de recherche agricole de Guinée (IRAG), Guinea;
Simplice Koffi Kouassi, Centre national de recherche agronomique (CNRA), Côte d’Ivoire;
Lawrence Aboagye, Plant Genetic Resources Centre, Niaouli, Ghana;
Flore Sindemion, Institut national de recherches agricoles du Bénin (INRAB), Benin;
Antoine Mputu Kena Kudia, Institut national pour l’étude et la recherche agronomique (INERA), DRC;
Clotilde Ngnigone Ella, Institut de recherches agronomiques et forestières (IRAF), Gabon;
Fernand Mouketo, Centre de recherche agronomique de Loudima (CRAL), Congo;
Selome Y. Dogbe, Institut togolais de la recherche agronomique (ITRA), Togo;
Olagorite Adetula, National Horticultural Research Institute (NIHORT), Nigeria;
Robert Muhwezi, National Agricultural Research Organization (NARO), Uganda;
Mkulila Shaban, Agricultural Research and Development Institute (ARDI), Tanzania;
Margaret Ongango, Kenya Agricultural Research Institute (KARI), Kenya;
Antoine Nsabimana, Institut des sciences agronomiques du Rwanda (ISAR), Rwanda; Ferdinand Ngezahayo, Institut de recherches agronomique et zootecniche (IRAZ), Burundi; Dickson L.N. Banda, Department of Agricultural Research and Technical Services, Malawi; Dejene Abera, Ethiopian Agricultural Research Organization (EARO), Ethiopia; Connie Fraser, Institute for Tropical and Sub-Tropical Crops (ITSC), South Africa.

From regional/international organizations

William Ngeafack, Centre africain de recherches sur bananiers et plantains (CARBAP), Cameroon; Chyka Okarter and Perpetua Udu, International Institute of Tropical Agriculture (IITA), Onne research station, Nigeria; Emmanuel Njukwe, International Institute of Tropical Agriculture (IITA), Mbalmayo, Cameroon; Deborah Karamura, INIBAP-ESA, Mbalmayo, Cameroon; Elizabeth Arnaud and Suzanne Sharrock, INIBAP Headquarters, France; Xavier Perrier, CIRAD, France.

Resources persons:

Kodjo Tomekpe CARBAP, Cameroon; Ekow Akyeampong, INIBAP – MUSACO, Cameroon; Emmanuel Njukwe, International Institute of Tropical Agriculture (IITA), Mbalmayo, Cameroon; Deborah Karamura, INIBAP-ESA, Uganda.

Geographical Information Systems (GIS) and Musa diversity

INIBAP is investigating the possibility of using a newly developed GIS software (DIVA) for the spatial analysis of Musa genetic resource information. DIVA has been developed by the International Potato Centre (CIP) with the support of FAO, the CGIAR’s System-wide genetic resources programme (SGRP) and IPGRI. A training course was held recently at INIBAP’s headquarters in Montpellier to train staff in the use of the software and to assess the potential to use this software in association with data available in the Musa Germplasm Information System (MGIS), in particular with data recorded during collecting missions. INIBAP hopes to use the DIVA-GIS software for the following tasks:

- Identification of where gaps exist in the coverage of collecting missions (comparison with production areas);
- Documentation of the potential effects of climate change and other potential genetic erosion factors on wild species distribution;
- Comparison of species distribution in relation to pests and diseases;
- Predict and assess impact, e.g. of new varieties.

More information about the DIVA software is available from the CIP website (http://www.cip.org/diva/). The software is freely available and can be downloaded, along with the user’s manual, from the website.

Fifth meeting of the regional steering committee of MUSACO

From 11 to 12 February 2002, the regional steering committee of the banana and plantain research network for west and central Africa, MUSACO, met in Cotonou, Benin for its annual meeting. In attendance were representatives from Benin, Cameroon, Congo Republic, Côte d’Ivoire, Gabon, the Democratic Republic of Congo, Ghana, Guinea, and Sierra Leone as were those of CARBAP (formerly CRBP) IITA and INIBAP. Togo was admitted as the 13th member of the network at this meeting.

In a speech to officially open the meeting, Dr David Arodokoun, Scientific Director of the Institut national des recherches agricoles du Bénin acting on behalf of his Director General stressed that banana and plantain are crops that could contribute to food and nutritional security, alleviation of poverty, and the creation of employment in Benin. Banana and plantain are two of the crops that Benin is now promoting to diversify the food crop base. He reported that 33% of households in Benin regularly consume plantains and Musa production in Benin has increased from 22,000 tonnes in 1998 to 45,000 in 2001. He hoped that the research carried out in the network will help find solutions to constraints facing farmers in Benin such as lack of clean planting materials, pests and diseases and post-harvest transformation of the fruit.

Adoption of the report of the Accra meeting

The first order of business was the adoption of the minutes of the last regional steering committee meeting held in Accra in April 2001. Before members would do that, they wanted to know if the recommendations of that meeting had been implemented. The President and secretary provided the following information on the various recommendations that were made in Accra:

- The training course on Mycosphaerella leaf spot diseases was held in Malaysia in June 2001. Drs Kobena Konuman of CNRA, Côte d’Ivoire and Ekow Akyeampong were the two participants from West and Central Africa. A second training course on the post-laboratory handling of tissue-cultured plantlets and on rapid multiplication of Musa planting materials was organized at CARBAP, Nyombé from 2 to 7 December 2001 for francophone member countries of the network.
- The nine countries which received funds from INIBAP have collected and sent the secondary baseline information to the network secretariat. A report is being prepared by a young professional officer seconded to the office by the FAO.
- Concerning participation in PROMUSA working groups by scientists from the sub-region, only Benin, Côte d’Ivoire, Gabon and Ghana sent names of

Participants to the 5th MUSACO meeting.
researchers to the PROMUSA secretariat through Dr Adiko, the West and Central Africa representative on the steering committee of PROMUSA.

- As at the time of the meeting, nothing had been heard from CORAF regarding the eight initiatives written in collaboration with CARBAP that were sent in response to its call for projects.
- On the question of IITA scholarships, members were informed that Mr Ben Banful, former representative of Ghana on the MUSACO steering committee has received an IITA scholarship for PhD studies.

Country Musa news

Each country representative gave a brief report on new activities that are taking place in their countries. To popularize banana and plantain production in Sierra Leone, nurseries and demonstration plots have been established in four districts. These will be extended to four more districts. Also, local varieties have been collected and planted for characterization purposes.

As in Sierra Leone, to re-launch plantain and banana production in Littoral and in the Forest regions of Guinea (Conakry), nurseries have been established to produce clean planting materials that will be distributed to farmers. Among the varieties to be given to farmers are hybrids of IITA and CARBAP.

Studies are being planned in Côte d’Ivoire to attempt to explain the observation that Pratylenchus spp. seems to be replacing Radopholus similis as the main nematode on Musa. IITA is interested in the issue of changes in species composition and will consider offering a doctoral fellowship for work on this in collaboration with KULeuven. It was recommended that a nematological survey be conducted in all member countries to determine if the nematode diversity and the relative abundance of the species remain the same as before.

The other new activity in Côte d’Ivoire was the high planting density technology that Ivorian scientists are testing on-station. The high planting density trials being conducted in Côte d’Ivoire and also in Cameroon are the follow-up of a visit made by ten scientists, farmers and extension agents from West and Central Africa to the Dominican Republic and Costa Rica to study the high density plantain plantation production technologies being used there. The delegation was impressed by the up to 60% yield increases that have been obtained in the Latin America and Caribbean region from managing ‘False horn’ plantain as an annual crop at densities from 2500 to 5000 plants per hectare.

Ghana’s new representative on the steering committee, Dr Anno-Nyako informed the meeting that Musa breeding has started in Ghana. A plant biology laboratory with a tissue culture unit has been established in Gabon.

Being the first time to participate in the meeting, the Togolese representative, Dr S. Dogbe, gave an overview of the research on banana and plantain in his country. Bananas and plantains are cultivated in the cocoa/coffee zone as a shade for the cash crops. The programme has a field genebank consisting of 32 accessions many of which originate from Ghana. A major problem confronting the production of the crops in Togo is the lack of good quality planting materials.

Updates on regional projects

Members reported on the two regional projects, Musa germplasm evaluation and periurban Musa production.

Germplasm evaluation trials have been established in almost all the network countries but it is only in Côte d’Ivoire that the first crop has been harvested. It was reported that ‘FHIA-23’ is the most productive among the bananas (‘FHIA-01’, ‘FHIA-18’ and ‘SH-3460’) that are being evaluated in Côte d’Ivoire, but it also has the longest crop cycle. The inoculum pressure of black leaf streak disease (BLSD) was so high that at harvest, ‘Orishele’, the susceptible plantain variety had virtually no functional leaves. Consequently, bunch weights of ‘Orishele’ were low compared to the resistant hybrid, ‘CRBP-39’, which maintained six green leaves to harvest. Tests to determine consumer acceptance of the fruits are being conducted but the results are not yet available.

The periurban Musa production project is making good progress in Benin with the vitroplantlets provided by CARBAP. Among the hybrids established in Benin are ‘FHIA-25’, ‘FHIA-18’, ‘FHIA-23’ and ‘CRBP-39’. Survival of plants in the field was very high so is the enthusiasm of the 40 farmer participants. The visit to some of the farms generated a lot of interest as the plants were doing very well. The researchers and farmers exchanged views on farmer participatory research approaches. These discussions continued when the scientists returned to the meeting place the next day. A survey on periurban Musa production conducted in Benin revealed that 56% of Musa farmers cultivate plantain on an average plot size of 0.8 ha. In Benin also, inadequate supply of planting materials is cited a bottleneck to expanded production.

The periurban project has not really taken off in Ghana as the national institution that was supposed to supply the planting materials failed to do so. Materials imported from South Africa have been weaned and will be supplied to farmers at the beginning of the rainy season.

International and regional institutions

CARBAP and IITA each sent a delegation of several scientists to present the different areas of Musa research at their centres. Dr Lutaladio representing FAO talked about collaboration with the network.

CARBAP presented the advances of their programmes regarding plantain breeding, agronomy and integrated systems, plant pathology and pest control with an integrated pest management approach (leaf spot diseases, nematodes and weevil), post-harvest technologies and socio-economics. Achievements of the centre include the development and transfer of in vivo multiplication techniques to produce large numbers of clean planting materials, and ‘CRBP-39’, a plantain-like hybrid that has been released and distributed within the MUSACO network and to more than 40 countries around the world in the framework of the 3rd International Musa Testing Programme coordinated by INIBAP. Secondary triploids with resistance to diseases have been created by different breeding schemes and short and early yielding plantain hybrids are also under evaluation. Banana and plantain-based...
snack foods, infant formulas and flour have been developed.

Presentations from IITA were on participatory research, integrated pest management, Musa breeding and agronomy. In farmer participatory evaluations conducted in eastern Nigeria, farmers preferred ‘PITA-14’ one of IITA’s plantain-type hybrids over ‘Agaabga’, the local variety. ‘PITA-14’ gave higher financial returns as well. The meeting was informed of a pilot project supported by USAID in which hybrids developed at CARBAP, IITA and FHIA are being evaluated in farmers’ fields in Nigeria. It is hoped that after the pilot phase, the project will be expanded to other countries in the West and Central Africa. IITA continues to develop plantains hybrids with superior resistance or tolerance to diseases, including a practical physiological-genetic and biotechnological approach to control banana streak virus, and good agronomic characteristics such as earliness, short stature, and good rooting. Integrated pest management research has included developing and testing methods such as using hot or boiling water to clean sucker's contaminated with pests. There are also studies to determine the efficacy of nematocidal plants such as Flemingia interplanted in plantain fields.

The four projects of INIBAP, namely germplasm management, germplasm improvement, information and communications and regional networks were briefly described and activities in Africa under each were mentioned. The objectives and modus operandi of PROMUSA, the global programme for Musa improvement, coordinated from INIBAP headquarters were also described. The FAO representative at the meeting, Dr Lutaladio, traced the history of the collaboration between his department and INIBAP. In 1999 AGPC/FAO and INIBAP discussed collaboration on the gathering and exchange of information and the transfer of technology. A young professional officer (YPO) was recruited and posted to the INIBAP/MUSACO secretariat to develop instruments for the collection and compilation of baseline information and to incorporate collected information into HORTIVAR, a database on performances of horticulture cultivars in relation to environmental conditions and cultivation practices. In addition, the YPO was to provide inputs in the urban and periurban horticulture programme in relation to food security and was to assist in project proposal development. Dr Lutaladio informed the meeting that the YPO has prepared preliminary reports on the different tasks he was given at the beginning of his assignment.

In the immediate future the FAO will assist at least two MUSACO member countries in designing projects for improvement of banana and plantain production for small-scale growers through the setting up of efficient and cost-effective multiplication systems for production of disease-free planting materials. The FAO will collaborate with MUSACO, CARBAP and IITA to collect and characterize Musa germplasm in the Congo basin, to upgrade tissue culture and nursery facilities in certain countries, and to train researchers in handling tissue culture plants, virus indexing, and rapid production of planting materials. Finally, the FAO will assist in the development of a protocol for mass propagation and distribution of quality planting materials.

Functioning of the network

The delegates discussed ways to improve the operation of the network. For a start, it was agreed that working groups based on identified research priorities should be set up. These will meet as often as necessary depending on available resources. Working groups are to be formed immediately on (1) rapid multiplication of clean planting materials; (2) profitable plantain production systems and (3) farmer participatory research. The secretary of the network was asked to identify leaders for each of the three working groups. Activities proposed by the working groups will be approved and their implementation monitored by the regional steering committee. The current unsatisfactory communication links among members and between members and the secretariat was attributed to the lack of information technology equipment in many of the research stations where the Musa programmes are based.

Recommendations

The assembly passed the following recommendations:

1. A survey should be conducted in all member countries to determine the diversity and prevalence of Musa nematodes in West and Central Africa;
2. INIBAP, IITA and CARBAP should assist MUSACO to organize training courses on germplasm evaluation and on participatory research methods;
3. All should wait for results on the high-density planting technology trials being conducted by CNRA in Côte d’Ivoire and CARBAP in Cameroon before disseminating the technology to farmers;
4. Working groups on (a) rapid multiplication of clean planting materials; (b) profitable plantain production systems and (c) farmer participatory research should be created immediately within the network;
5. A grant proposal should be developed to look for funds to equip all national programmes with basic information technology equipment and Internet access.

MUSACO 2003

The next steering committee meeting will be hosted by IRAG in Guinea (Conakry) during the first week of March 2003, under the presidency of Mr Bernadin Lokossou, the representative of Benin who was elected to replace Madam Adèle Sambo of Gabon.

2nd International workshop on Mycosphaerella leaf spot diseases of bananas

San José, Costa Rica, 20–23 May 2002

This workshop was organized by INIBAP in collaboration with the Corporación Bananera Nacional (CORBANA), the Escuela de Agricultura de la Región Tropical Húmeda (EARTH) and the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE). Coming 13 years after the last international meeting on this topic, it provided a timely opportunity to analyse the current situation regarding Mycosphaerella leaf spot diseases at the global level. The meeting also allowed new lines of investigation to be suggested and facilitated the re-orientation of breeding programmes and biotechnology strategies for the genetic improvement of bananas and plantains.

More than 60 scientists attended the workshop from both the public and private sectors, representing more than 16 different countries from Latin America and the Caribbean, Europe, Africa, Asia and the Pacific, including Australia.

In order to maximize the outputs of the meeting and to guarantee the development of new strategies in the control of the different Mycosphaerella leaf spot diseases, participation in this workshop was by invitation only. The results of the meeting will however be widely disseminated through the publication of the proceedings.

The meeting was inaugurated by Dr Jorge Sauma, Director of CORBANA. Dr Emile Frison, Director of INIBAP, welcomed all the participants and paid tribute to Ramiro Jaramillo Celis, former INIBAP regional coordinator for Latin America and the Caribbean, in recognition of his invaluable contribution and tireless efforts to the development of the
The workshop was organized around five main topics: 1) Impact of Mycosphaerella leaf spot diseases of bananas; 2) Population biology and epidemiology; 3) Host-pathogen interactions; 4) Genetic improvement for a management of durable resistance and 5) Integrated disease management.

During the workshop, participants had the opportunity to learn about the distribution and impact of the different Mycosphaerella leaf spot diseases in several countries around the world. Discussions were held at the end of each session to allow research priorities and corresponding activities required at the global level to be identified or refined, in order to significantly reduce the impact of these diseases and thus make Musa a more sustainably productive crop.

**Session 1. Impact of Mycosphaerella leaf spot diseases of bananas**

Introductory papers presented information on the global spread, current distribution and impact of the three Mycosphaerella leaf spot pathogens - *M. muscicola*, *M. fijiensis* and *M. eumusae*. Other papers described techniques developed in Australia for the rapid diagnosis of *M. muscicola* and *M. fijiensis*, the effects of *M. muscicola* and *M. eumusae* on banana cultivation in South Africa and the impact of *M. fijiensis* in Cuba, Brazil and tropical Asia. The latest taxonomic work undertaken on the anamorph of *M. eumusae* and on other Mycosphaerella species was also described. *M. fijiensis* continues to spread to new areas. From 2000 to 2002, the pathogen was identified for the first time in Madagascar, the Bahamas, and the Galapagos Islands of Ecuador and in the north Queensland banana growing area where eradication is being attempted. *M. eumusae* leaf spot has also been observed on *Mysore* (AAB) in Sri Lanka. As this clone has strong resistance to *M. muscicola* and *M. fijiensis*, this is the cause of some concern.

It was agreed that more taxonomic information about *Mycosphaerella* spp. is needed, as well as information on other related genera that either form or occur in banana leaf lesions. A greater knowledge of *Mycosphaerella* pathogens /saprophytes and those in related genera is a prerequisite to the development of rapid diagnostic tests to distinguish leaf spot pathogens. The exact distribution of *M. eumusae* also needs to be investigated. Further surveys in South and Southeast Asia to determine where *M. muscicola*, *M. fijiensis* and *M. eumusae* occur are necessary. More information on the effect of *M. eumusae* on the growth and yield of banana clones is needed. Information suggests that Cavendish and Plantain cultivars are very susceptible.

**Session 2. Population biology and epidemiology**

Pathogenicity and distribution variability, sources of resistance, epidemiology and population structure of the main species (*M. fijiensis*, *M. muscicola* and *M. eumusae*) at the national, regional and international levels were defined as fundamental information for the continued success of banana production. Such studies are particularly necessary in Asia, which is the centre of diversity of the three pathogens and where little research has so far been conducted. A study of the evolution of host-pathogen relationships for the three pathogens, particularly involving resistant cultivars, is of a special concern, in order to identify pathogen populations that could break down plant resistance and to evaluate selection pressure. Molecular tools such as microsatellites have been recommended to monitor the genetic variability of the pathogen populations and pathogenicity should be evaluated concurrently. Epidemiological studies, including disease dispersal, are needed to better understand the distribution and the spread of the pathogen and will complement, together with the studies on pathogenicity and genetic variability, all the information required to anticipate the evolution of pathogen populations and to define resistance management strategies.

**Session 3. Host-pathogen interactions**

Several cases of an unexpected level of susceptibility to black leaf streak disease (BLSD) have been reported. Although different reasons have been offered to explain the phenomenon (poor nutrition, environmental stress), the problem of the erosion of resistance cannot be ignored and requires a precise characterization of the pathogen population. A greater understanding of the mechanisms involved in plant-pathogen interactions continues to be needed to ensure the long term success of breeding programmes. Further studies are also needed to compare the effect of infection by each of the three pathogens (*M. fijiensis*, *M. muscicola* and *M. eumusae*) on the host plants. Other pathosystems (such as Magnaporthe grisea) have shown the powerful nature of the genetic approach to identify without any a priori the pathogenicity factors. These approaches include the study of gene expression during production of pathogenicity mutants, comparative genomic and gene function validation techniques. Consequently, the development of genetic and molecular biology tools for *M. fijiensis* in collaboration with *M. graminicola* groups and the launching of a genomic initiative to access genomic tools and set up a genomic-wide comparison of *M. fijiensis* with *M. graminicola* have been recommended.

It is also recommended that the different mechanisms of resistance (partial or vertical) should be studied.

**Session 4. Genetic improvement for a management of durable resistance**

During this session, progress that has been made towards the creation of new varieties resistant to BLSD, either...
through conventional and/or modern technologies, was presented. New tetraploids hybrids resistant to BLSD are already available and some of these are widely grown around the world. However, because of the lack of new sources of resistance and due to the presence of the activable form of the banana streak virus (BSV) in interspecific hybrids (A x B), the production of a new generation of triploid hybrids is seriously jeopardized. Good progress has been reported in the development of a molecular toolbox for bananas and plantains in the area of the genetic transformation, allowing the production of transgenic banana plants.

The study of the diversity of the Musa balbisiana genome, using both morphological and molecular characterization was the first recommendation from this session. It was also recommended, that in anticipation of the needs of genetic improvement programmes, the T and S genomes, Musa textilis and Musa schizocarpa respectively, should also be screened. Mutation induction techniques should no longer be seen as an independent genetic improvement strategy but more as a tool that can contribute to cross-breeding programmes by increasing genetic diversity of parental lines. Mutants could also help in understanding the mechanisms of resistance (functional genomics).

**Session 5. Integrated disease management**

Yellow and black Sigatoka control strategies on banana can, according to the country and the scale of production, include not only chemical and cultural practices but also the use of mixed crops or resistant clones. The important inhibitory effect of some natural substances derived from microorganisms antagonistic to fungi, have also been reported as effective in reducing the development of M. fijiensis in vitro.

The integration of various or specialists from different disciplines has been recommended to facilitate the development of an achievable integrated pest management (IPM) approach for banana leaf spot diseases. The participants in the workshop also recommended to investigate the potential of natural or synthetic substances able to promote or activate systemic acquired resistance in its broadest sense.

The proceedings of the workshop will be published shortly by INIBAP. This publication will include the full papers of all presentations and a summary of the discussions and recommendations. It is expected that the proceedings will become a reference document of Mycosphaerella diseases for the next ten years.

**Books etc.**

**Strategy for the Global Musa Genomics Consortium**

Report of a meeting held in Arlington, USA, 17-20 July 2001
ISBN: 2-910810-48-08

The Global Musa Genomics Consortium was launched at a meeting held in Arlington, Virginia, which was critical for laying a solid foundation for future collaboration in Musa genomics and allowed the first important steps to be taken towards the development of a coherent strategy for Musa genomics.

This document provides further background information about the establishment of the Consortium and its aims and objectives. It also provides a review of the current status of Musa genomics research and provides details of the nature and scale of the work to be carried out by the Consortium members. Information is provided on an incremental strategy developed by the Consortium to achieve its goals and the proposed modus operandi, as agreed during the Arlington meeting. Further details are provided in the Annexes.

Copies are available from INIBAP Headquarters.

**Addendum to the ‘Descriptors for Banana (Musa spp.)’**

To complete the revised version of the ‘Descriptors for Banana, Musa spp.’ and responding to demand from East Africa, additional characters specific to the East African Highland bananas were incorporated as an addendum in 2001. The descriptors for Musa are unique in including a colour chart. This helps to remove the subjective nature of colour recording, and leads to a common understanding of such characters.

Copies of the descriptors and their addendum are available from INIBAP Headquarters.

**Resúmenes analíticos de la investigación sobre plátano en Colombia**

Edited by D.G. Cayón and F. Salazar Alonso
ISBN: 958-96885-1-9

CORPOICA could certainly be proud for the effort they made recently to identify, analyse and compile the existing information on plantain research and technology transfer in Colombia under the title ‘Resúmenes Analíticos de la Investigación sobre Plátano en Colombia’. This information product is a unique inventory of the major part of the scientific literature published on this topic including grey literature. It includes 792 abstracts and authors- and thematic indexes which make the search easier for the reader.

This important document in Spanish is available in both electronic (database on CD-Rom) and printed (400 pages) formats and will certainly be highly appreciated by all those working on plantain at international level.

The document is available on request at CORPOICA, Apartado Aéreo No 1087, Av Bolivar Sector Regìvit 28 Norte, Armenia, Quindío, Colombia – Fax: (57-6) 7496331 and at the INIBAP Office for Latin America and the Caribbean, C/o CATIE, 6170 Turrialba, Costa Rica.

**Banana varieties: The ACIAR years 1987-1996**

J.W. Daniells and N.J. Bryde
Information series Q101013 ISBN 0727-6273

During the period 1987-1996, the Queensland Department of Primary Industries (QDPI) was the lead agency of the ACIAR project ‘Banana improvement in the South Pacific’. In the course of the project, banana varieties were collected from all around the world. One hundred
and six varieties are reported in this publication which represent significant cross-section of those available for evaluation. They include some hybrids from conventional breeding programmes, selections originating as off-types from tissue culture propagation, existing cultivars and wild species.

The report put together the agronomic information collected along with colour photographs of bunches which permit a good appreciation of each variety. Many readers will also find these photographs useful for identification purposes.

Clean & green bananas - Where to from here?
J.W. Daniells
Information series Q101014
ISBN 0727-6273
Current sales for clean & green/organic bananas are very limited in Australia. Organic export is risky and uncertain. However, the market is shifting in this direction and in the longer term, the organic niche will probably eventually grow to 10-15% of the market. Major sales of both products will be facilitated by supermarket participation and the current price of the existing organic product will need to come down. Efficiencies of production must be improved by specific research on limiting factors such as soil fertility management, leaf disease control and greater development/extension/adoption of existing technology. Good progress has been made by the banana industry to reduce pesticides use but it is now necessary to pull together the body of knowledge and develop an ECO-OK type system implemented on commercial farms which complies to auditable standards and market development so that producers are rewarded for their efforts.

The two publications mentioned above are available on request at Department of Primary Industries, GPO Box 46, Brisbane QLD 2001, Australia.

**Announcements**

3rd International symposium on the molecular and cellular biology on Musa
9-11 September 2002, Leuven, Belgium
The first symposium, held in March 1999 at Cornell University in Ithaca, USA, was organized to open a forum where all those interested in molecular and cellular biology had the opportunity to meet and exchange ideas about their research activities. The meeting was a resounding success. It was therefore suggested that this concept be continued under the auspices of PROMUSA. After the 2nd International symposium on the Molecular and cellular biology of bananas held in October 2001 in Byron Bay, Australia, INIBAP and KULeuven are pleased to announce the 3rd International symposium on the Molecular and cellular biology of bananas, held in Leuven, Belgium from the 9th to the 12th September 2002.

The scientific programme includes five sessions. During the event, more than 30 papers and 31 posters will be presented. Twelve keynote lectures will also be given by outstanding speakers:

- Prof. Francis Quétier, Dr Xavier Draye and Prof. Guido Volkaert (Session 1: Genomics),
- Drs A. De Picker and D. Inzé (Session 2: Gene expression and transformation),
- Prof. G. Gheysen, Drs Johan Nayts and D. Van Der Straeten (Session 3: Molecular plant pathology and disease/pest resistance),
- Drs Isabel Roland-Ruitz and Peter Breyne (Session 4: Biodiversity characterization and conservation) and Drs Emma Schofield and W. Peumans (Session 5: Biochemistry and physiology).

A workshop on Intellectual property and genetically modified organisms will also be led by Dr Victoria Henson.

A scientific visit to KULeuven and the INIBAP Transit Centre facilities will be organized.

To know more about the symposium, visit the INIBAP web site at:

http://www.inibap.org/actualites/actualites_eng.htm

Global conference on banana and plantain
Grand Ashok Hotel, Kumara Krupa,
High Grounds, Bangalore, India
October 28 to 31, 2002
To address the new emerging agenda for growth and development of the banana industry, the Association for the Improvement in Production and Utilization of Banana (AIPUB), India, with support of INIBAP, the Food and Agriculture Organization of the United Nations (FAO), Department of Agriculture and Cooperation, Government of India and Indian Council of Agricultural Research, is organizing a global conference on banana and plantain focusing on 'Banana production for nutrition and livelihood security'.

This Conference is being organized with the following objectives:
- To bring together global players in banana research, development and trade for deliberation and discussion on various issues for sustainable development of banana.
- To deliberate upon the opportunities for Indian banana and banana products in international trade.
- To involve national and international experts to develop and recommend policy initiatives for nutritional and livelihood security through banana production.

The Conference deliberations will focus on the following topics:
- Genetic resource management and crop improvement;
- Biotechnological advances;
- Strategies in production technology;
- Organic production of banana;
- Integrated disease and pest management;
- Post harvest management, product diversification and value addition;
- Policy support and programmes;
- National and international trade;
- International cooperation.

**Important dates**
Deadline for submission of abstracts - 31st July 2002,Deadline for submission of full papers - 30th September 2002

**Registration fees**

Before 30 August 2002
- Member of AIPUB: 50 US$
- No member: 75 US$
- Corporate: 100 US$

After 30 August 2002
- Member of AIPUB: 100 US$
- No member: 125 US$
- Corporate: 150 US$

Registration form along with the fees should reach the Conference Secretariat:
Bagwani Bhavan, 47 Jankapuri Institutional Area, Pankha Road, New Delhi-110058; Tel (91-11) 5622150/5531211; Fax (91-11)-5531211/33849780.

For more information and registration, visit the web site:
http://www.aipub.org/conferences.htm
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• Regional Office for Asia and the Pacific
Regional Coordinator
Dr Stover R.H. & N.W. Simmonds.
Books

• References:
Articles (or chapters) in books
Musa breeding at

• Acronyms:

• Abstracts:

• Instructions to authors

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

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

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

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

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

Please follow the style shown below:
Periodicals:

Books:

Articles (or chapters) in books:

Tables: These should be numbered consecutively and referred to by these number in the text. Each table should include a title.

Illustrations: These should be numbered consecutively and referred to by these numbers in the text. Each illustration should include a clear and simple caption. Graphs: provide the corresponding raw data with the graphs.

Drawings: provide originals if this is possible. Black and white photographs: provide them on bright paper and with good contrast. Colour photographs: provide good quality proofs and films or original slides.

Note: When plant material used for the experiments reported originales or is registered in the INIBAP genebank, its accession number (ITC code) should be indi-

Thank you in advance for following these instructions
This will facilitate and accelerate the editing work.
The meeting started with a brief presentation by each participant of their research capacity, in terms of human resources, research facilities, and future and ongoing projects related to *Mycosphaerella* leaf spot diseases. Participants also commented on their participation in PROMUSA, defining their areas of interest where they would like to develop partnerships with other participants.

The participants identified various research priorities and defined the main activities that should be carried out.

## Recommendations

### Development of a detailed understanding of the population structures of *Musa* species, *M. fijensis* and *M. eumusae*

#### Survey of geographical distribution of the three *Mycosphaerella* species

The survey of the distribution of the different species requires wide sampling at the national level of the different agroecological areas where *Musa* is found, and the morphological characterization of the species through the observation of the anamorph stage (conidia), including molecular characterization using PCR diagnostics.

#### The PROMUSA Sigatoka working group ratified the recommendation made during the “2nd International workshop on Mycosphaerella leaf spot diseases of bananas” held from 23 to 25 May 2002 in San Jose, Costa Rica: “The exact distribution of *M. eumusae* needs to be known. Further surveys in south and southeast Asia to determine where *M. musicola*, *M. fijensis* and *M. eumusae* occur are necessary. The name of the banana clone affected, an indicator of the severity of the leaf spot and local environmental data would be useful as this may help explain distribution. IMTP trials are seen as ideal locations for assessing the reaction of different clones to the different leaf spot pathogens. The collection and diagnosis of specimens of leaf spot from IMTP trials sites needs to be continued. The cooperation and collaboration of scientists in south and southeast Asia is viewed as essential. Identification tools should be provided to enable diagnoses to be undertaken locally”.

#### Development of national collections of the different *Mycosphaerella* leaf spot pathogens

The PROMUSA Sigatoka working group ratified the recommendation made during the “2nd International workshop on Mycosphaerella leaf spot diseases of bananas”: A reliable, rapid test to distinguish *M. musicola*, *M. fijensis*, *M. eumusae* and possible other *Mycosphaerella* pathogens/epiphytes needs to be developed to aid identification. Information on how to distinguish the 3 pathogens on morphological characteristics also needs to be produced and circulated to banana scientists. INIBAP was asked to address this need.

The creation of a national collection of different strains from different *Mycosphaerella* leaf spot pathogens on *Musa* is of special relevance in the understanding of the population structure. The collection must be based on single-ascospore cultures with an *in vitro* characterization of the anamorph stage (in vitro sporulation of conidia). It has
been recommended to provide the participants with a protocol to sample, establish and maintain the collection. INIBAP was mandated to collaborate with CIRAD in the development and distribution of the technical information required. The establishment of a national collection should be promoted and facilitated through the organization of a training course; especially for those countries that develop breeding programmes, but also where disease resistant hybrids of banana are used on an industrial scale, and where the high diversity of Musa would have originated similar diversity in the pathogens.

**Genetic population structure**

The study of the genetic population structures of Mycosphaerella leaf spot diseases is already on going at national, regional and international levels. However, the group recommends increasing the number of countries involved at the national level, which will allow refinement of both regional and international studies. Both biological (morphological) and molecular determinations have been recommended to improve the understanding of the different population structures. The sampling protocol and methodology should be standardized and the recognition of the different species facilitated through the development of a technical factsheet to be widely distributed. INIBAP and CIRAD agreed to work together in the preparation of this information which should include several detailed illustrations of the different pathogens and their anamorph stages. This information will also become part of the IMTP guidelines. The development of more molecular markers as SSR and CAPS should allow the study of the different populations to be refined. The recommendation to include partners from south and southeast Asia made during the last global meeting of PROMUSA in Bangkok was reiterated by the participants who strongly suggested that the INIBAP regional office for Asia and Pacific strengthen and facilitate any exchange between Asian partners and the rest of the PROMUSA community.

**Pathogenic characterization**

The pathogenicity of the different strains should be approached using either the in vitro or in vivo inoculation systems. However, it is recommended that the different methodologies that currently exist be standardized. The methodology for the in vitro inoculation on leaf fragments developed at CIRAD should be distributed, together with the methodology used to isolate, cultivate and produce the inoculum of the different pathogens. INIBAP and CIRAD have been requested to compile, in a single technical document, all the different information already published on these different methods.

### Identification of new sources of resistance

The need of new sources of resistance to Mycosphaerella leaf spot diseases has been identified several times in the past. Collecting missions in Indonesia, north India and Vietnam have already taken place but information has only been provided on the characterization of the resistance of the different materials collected. The PROMUSA working group recommended that INIBAP help to gather any information already available. The group also recommended to stimulate the characterization of existing collections where M. eumusae has already been reported together with other Mycosphaerella spp. e.g. MARDI, in Malaysia. In order to facilitate the screening, the group suggested using the ‘severity index’ as the unique parameter to detect any source of resistance. This information should allow the definition of the different reference clones needed to evaluate the resistance to Eumusae leaf spot disease.

The ‘severity index’ will also be used to evaluate the PROMUSA segregating populations hosted at CORBANA.

### Diagnostics

Several leaf fungal diseases have been reported on Musa and other related species. The group recommended the development of specific diagnostic tools according to the three main species of Mycosphaerella pathogen on Musa: M. fijiensis, M. musicola and M. eumusae. The achievement of these diagnostic tools will remain within the development of a worldwide collection of Mycosphaerella isolates; the morphological description of all the different Mycosphaerella subspecies associated with banana leaves and the development of primers species-specific as microsatellites and ITS-sequences and their test on the worldwide collection of Mycosphaerella isolates.

It is therefore suggested:

- to develop diagnostic tools to distinguish the main pathogens and assess currently available molecular methods for specificity,
- to develop a manual with descriptions of symptoms and morphological characters,
- to develop protocols for collection and analysis of samples,
- to transfer to and train PROMUSA participants on the different technologies required (collection and sampling, monascosporing cultures and molecular markers).

### Durability of the resistance

Significant changes in the levels of resistance to Sigatoka and black Sigatoka diseases have been reported in Australia, India and Cuba. However these may exist just because of high inoculum loads. Thus, changes in pathogen populations should be distinguished from particular epidemiological effects. Therefore, the group recommended studying the changes in pathogen populations in response to selection pressure from new banana genotypes resistant to Sigatoka diseases. It is essential to monitor changes in pathogen populations in areas where new resistant hybrids are being grown on a large scale. A special recommendation towards the development of specific trials in Cuba has been made. Two different aspects of the durability of the resistance need to be addressed: the genetic drift of the pathogen resistance and the selection effect within the pathogen population. Participants in PROMUSA Sigatoka working group recommended:

- the selection of areas where resistant hybrids have been grown for a long time (e.g. Cuba) and to follow the evolution of the pathogen populations, isolating Mycosphaerella strains on resistant and susceptible cultivars or hybrids,
- the development of molecular markers linked to the pathogenicity of the fungal strains (molecular markers will inform on the genetic drift when pathogenic evaluation will be related to the selection effect),
- the quantification of the selection pressure over the time, and
- the study the breakdown of resistance by in vitro testing.

### Dispersal of Mycosphaerella leaf spot diseases on Musa

*M. eumusae* is currently limited in extent throughout most of Asia, although there is some evidence that the pathogen may have reached Africa. The dynamics of the disease are not fully understood. Some projections indicate that this disease will become more important than black Sigatoka. In order to prepare adequate disease control strategies, a detailed knowledge of the epidemiology of this pathogen is urgently required. To address the epidemiology of the different Mycosphaerella spp. pathogens on Musa, the group recommended:

- the collection of disease incidence data from the field and literature,
• the development of methodologies to understand the mechanisms of spore release and spore survival in the air at laboratory level, and
• the clarification of laboratory data at plantation level and to assess the potential for windborne dispersal (as opposed to further spread of the disease by the transfer of inoculum).

Host-pathogen interactions
The genetic approach has been shown to be extremely powerful when studying host-pathogen interactions in some pathosystems (such as *Magnaporthe grisea*). This approach does not require the identification of pathogenicity factors *a priori* and includes the study of gene expression during infection (differential display, DNA chip, SSH, etc.), production of pathogenicity mutants, comparative genomic and gene function validation techniques.

Here again, the PROMUSA Sigatoka working group ratified the recommendation made in the framework of the “2nd International workshop on Mycosphaerella leaf spot diseases of bananas” to study: “the development of genetic and molecular biology tools for *M. fijiensis* in collaboration with *M. graminicola* groups as well as to launch a genomic initiative to access to genomic tools (EST collection, physical map, genome sequence) and set up a genomic-wide comparison of *M. fijiensis* to *M. graminicola*”.

**International core collection**
The group recommended to develop an international core collection of *M. fijiensis*, *M. musicola* and *M. eumusae*. The different strains should be conserved as fungal mycelia and DNA. CIRAD was suggested to host the international collection using a similar mechanism as INIBAP developed with KULeuven to host the international Musa germplasm at the INIBAP Transit Center. INIBAP was asked to address this need in collaboration with CIRAD.

**Institutional contribution to PROMUSA**

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<th>On-going activities</th>
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<td>CORBANA, Costa Rica</td>
<td>Tissue culture lab.</td>
<td>Principal plant pathologist (1)</td>
<td>• Field evaluation on new clones</td>
<td>• IMTP phase III</td>
<td>J.A. Sandoval, R. Vargas</td>
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<td></td>
<td>Plant pathology lab.</td>
<td>Technical officers (2)</td>
<td>• Inoculation of <em>M. fijiensis</em> at greenhouse level</td>
<td>• Evaluation of Musa segregating populations</td>
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<td>Germplasm field bank Experimental fields</td>
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<td>• Epidemiology of <em>M. fijiensis</em> in hybrids resistant populations</td>
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<td>• Diversity and distribution of <em>M. fijiensis</em> (molecular and morphological characterization)</td>
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<td>INISAV, Cuba</td>
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<td>• Mycosphaerella leaf spot diseases</td>
<td>• Population survey of <em>M. fijiensis</em> (variability and distribution)</td>
<td>L. Pérez Vicente</td>
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<td>Principal plant pathologist (1)</td>
<td>• Black leaf streak: Cultivar evaluation &amp; diagnostics</td>
<td>• Diversity and distribution of <em>M. fijiensis</em> (molecular and morphological characterization)</td>
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<td>• Sigatoka: Diagnostics &amp; epidemiology</td>
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<td></td>
<td>• Isolate collection &amp; preliminary ID by symptomatology and morphology</td>
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<td></td>
<td>• Sequence analysis of ITS regions of Oceanic isolates</td>
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<td>• If needed, sequence of other suitable genes will be undertaken</td>
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<tr>
<td>CRCTPP, QDPI, Australia</td>
<td>Plant pathology labs Glasshouse Research stations</td>
<td></td>
<td>• Mycosphaerella leaf spot diseases</td>
<td></td>
<td>R. Peterson (QDPI)</td>
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<tr>
<td></td>
<td></td>
<td>Principal plant pathologist (1)</td>
<td>• Black leaf streak: Cultivar evaluation &amp; diagnostics</td>
<td></td>
<td>J. Henderson (CRCTPP)</td>
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<td></td>
<td></td>
<td>Technical officers (2)</td>
<td>• Sigatoka: Diagnostics &amp; epidemiology</td>
<td></td>
<td>K. Grice (QDPI)</td>
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<tr>
<td>Institution, Country</td>
<td>Research facilities</td>
<td>Human resources</td>
<td>Research topics</td>
<td>On-going activities</td>
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<tr>
<td>CORPOICA, Colombia</td>
<td>Molecular laboratory</td>
<td>Researchers (4)</td>
<td>Mycosphaerella leaf spot diseases</td>
<td>Characterization of the Colombian population of Mycosphaerella leaf spot</td>
<td>A. Gutiérrez Rojas</td>
</tr>
<tr>
<td></td>
<td>Plant pathology lab</td>
<td>Technicians (3)</td>
<td>Population structure and diversity</td>
<td><strong>Vol 11, N° 1</strong></td>
<td>S. Aponte</td>
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<td></td>
<td>Field stations (different altitudes)</td>
<td>MSc students (4)</td>
<td>Pathogens characterization</td>
<td>Morphological and molecular characterization</td>
<td><strong>S. Aponte</strong></td>
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<td></td>
<td></td>
<td>PhD students (4)</td>
<td></td>
<td>Morphological and molecular characterization</td>
<td><strong>S. Aponte</strong></td>
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<td></td>
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<td>Undergraduates (2)</td>
<td></td>
<td></td>
<td><strong>S. Aponte</strong></td>
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<td></td>
<td></td>
<td>Undergraduates (13)</td>
<td></td>
<td></td>
<td><strong>S. Aponte</strong></td>
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<tr>
<td>CICY, Mexico</td>
<td>Biotechnology labs</td>
<td>Researchers (4)</td>
<td>Genetic improvement using biotechnology</td>
<td>Construction and characterization of 2 genetic BiBAC libraries of 2 diploid bananas, and development of a transformation protocol using vacuum infiltration mediated by Agrobacterium, CONACyt, 3 years (Resp.: Dr. A. James)</td>
<td>A. Jam es</td>
</tr>
<tr>
<td></td>
<td>Biochemistry &amp; molecular</td>
<td>Technicians (3)</td>
<td></td>
<td>Genetic and physical mapping of M. fijiensis, CONACyt; 3 years (Resp.: Dr. D. Kaemmer)</td>
<td>D. Kaemmer</td>
</tr>
<tr>
<td></td>
<td>biology of plants</td>
<td>MSc students (3)</td>
<td></td>
<td>Screening of Calcutta IV BAC library for Resistance genes (submitted) (Resp.: Dr. D. Kaemmer)</td>
<td>L. Conde</td>
</tr>
<tr>
<td></td>
<td>Equipment facilities:</td>
<td>Ph.D students (4)</td>
<td></td>
<td>Construction of a BAC library of M. fijiensis (submitted) (Resp.: Dr. A. James)</td>
<td>L. Peraza</td>
</tr>
<tr>
<td></td>
<td>CHEF MAPPER</td>
<td>Undergraduates (2)</td>
<td></td>
<td>Agronomic evaluation of newly introduced banana and plantain cultivars in Mexico, and of mutant-induced plants (submitted) (Resp.: Dr. A. James)</td>
<td><strong>Dr. A. James</strong></td>
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<tr>
<td></td>
<td>Capillary Sequencer</td>
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<td><strong>Dr. A. James</strong></td>
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<td>CHEF DRII</td>
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<td><strong>Dr. A. James</strong></td>
</tr>
<tr>
<td>CIRAD, Montpellier</td>
<td>Phytopathology labs</td>
<td>Researchers (2)</td>
<td>Taxonomy and identification of Mycosphaerella spp.</td>
<td>Collection of Mycosphaerella spp. in Asia</td>
<td>J. Carlier</td>
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<tr>
<td></td>
<td></td>
<td>Technicians (3)</td>
<td></td>
<td>Pathogen populations structure at different scales</td>
<td>C. Abadie</td>
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<td></td>
<td>Greenhouse &amp; climatic chamber</td>
<td>Geneticist (1)</td>
<td>Collection</td>
<td>Population structure of Mycosphaerella/leaf spot pathogens in Asia</td>
<td><strong>J. Carlier</strong></td>
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<tr>
<td></td>
<td>In vitro evaluation facilities</td>
<td>Technicians (3)</td>
<td>Molecular diagnostic</td>
<td>Efficacy and durability of partial resistance</td>
<td><strong>J. Carlier</strong></td>
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<tr>
<td></td>
<td>Access to in vitro laboratory</td>
<td>Students (2-9)</td>
<td>Distribution of Mycosphaerella spp. in Asia</td>
<td>Characterization of new resistance sources</td>
<td><strong>J. Carlier</strong></td>
</tr>
<tr>
<td></td>
<td>Collection of about 2500 isolates</td>
<td></td>
<td>Pathogenicity</td>
<td>Genetic of resistance</td>
<td><strong>J. Carlier</strong></td>
</tr>
<tr>
<td></td>
<td>Laboratory of molecular biology</td>
<td></td>
<td>Pathogenicity</td>
<td>Evolution of pathogen population on resistant cultivars (large area of culture)</td>
<td><strong>J. Carlier</strong></td>
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<tr>
<td></td>
<td>(CAPS &amp; microsatellites, sequencers, genomic)</td>
<td></td>
<td>Other</td>
<td>Plant disease resistance studies</td>
<td><strong>J. Carlier</strong></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Genome and host-pathogen interaction studies</td>
<td>Collection (core collection, network, data base?)</td>
<td><strong>J. Carlier</strong></td>
</tr>
<tr>
<td>CATIE, Costa Rica</td>
<td>Molecular biology Lab.</td>
<td>Researchers (2)</td>
<td>Biological control and resistance</td>
<td>Studies of population structure of M. fijiensis</td>
<td>A. S. Riveros</td>
</tr>
<tr>
<td></td>
<td>Plant pathology Lab.</td>
<td>Technician (2)</td>
<td>induction</td>
<td>Biotechnology of Musa: tissue culture, bombardment protocols</td>
<td>G. Rivas</td>
</tr>
<tr>
<td></td>
<td>Biologic control Lab.</td>
<td></td>
<td>Bank of antagonistic bacteria and fungi</td>
<td>Submitted:</td>
<td><strong>G. Rivas</strong></td>
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<tr>
<td></td>
<td>Tissue culture lab.</td>
<td></td>
<td>Microbiological products with antifungal potential or elicitor molecule</td>
<td>Population structure of M. fijiensis in Dominican Republic.</td>
<td><strong>G. Rivas</strong></td>
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<tr>
<td></td>
<td>Particles bombardment</td>
<td></td>
<td>Screening of plants with antifungal or resistance induction</td>
<td>Presented by T. Polanco (IDIAF) to IAEA in January 2002. Partners: CATIE (Costa Rica) and CIRAD (France).</td>
<td><strong>G. Rivas</strong></td>
</tr>
<tr>
<td></td>
<td>Shade house</td>
<td></td>
<td>Botanical products with antifungal or elicitor molecule</td>
<td>Population structure of M. fijiensis in Honduras and Dominican Republic.</td>
<td><strong>G. Rivas</strong></td>
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<tr>
<td></td>
<td>Tools offered</td>
<td></td>
<td>Adaptation/development of a rapid and efficient screening method to evaluate black Sigatoka resistance.</td>
<td>Presented by G. Rivas (CATIE) to FINNIDA. May 2002. Partners: FHIA (Honduras), IDIAF (Dominican Republic) and CIRAD (France).</td>
<td><strong>G. Rivas</strong></td>
</tr>
<tr>
<td></td>
<td>M. fijiensis: Isolating protocols, DNA extraction, DNA amplification, DNA electrophoresis</td>
<td></td>
<td>Genetic transformation of Musa to introduce resistance to black leaf streak disease</td>
<td>Studies of population structure of M. fijiensis</td>
<td><strong>G. Rivas</strong></td>
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<tr>
<td></td>
<td>Use of molecular markers for studies of populations induction of resistance Pathogenesis tests</td>
<td></td>
<td>Transformation protocol in Musa</td>
<td>Biotechnology of Musa: tissue culture, bombardment protocols</td>
<td><strong>G. Rivas</strong></td>
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<tr>
<td></td>
<td>Musa tissue culture protocols Bombardment protocols</td>
<td></td>
<td></td>
<td>Population structure of M. fijiensis in Honduras. Project supported by INIBAP/FHIA. Partners: CATIE (Costa Rica) and CIRAD (France).</td>
<td><strong>G. Rivas</strong></td>
</tr>
<tr>
<td>Institution, Country</td>
<td>Research facilities</td>
<td>Human resources</td>
<td>Research topics</td>
<td>On-going activities</td>
<td>Contact</td>
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<tr>
<td>EMBRAPA, Brazil</td>
<td>Cooperative network of 3 EMBRAPA research centres including their laboratories and fields</td>
<td>Researchers (9)</td>
<td>• Integrated control of Mycosphaerella leaf spot diseases in Brazil including breeding</td>
<td>On-going:</td>
<td>Zilton Cordeiro, Maria de Jesus B. Calvacante</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Evaluation of genetic and pathogenic variability in M. musicola</td>
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<td></td>
<td></td>
<td>• Evaluation of resistance to black and yellow leaf streak diseases</td>
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<td>• Epidemiology</td>
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<td>New proposal:</td>
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<td></td>
<td></td>
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<td></td>
<td>• Creation and evaluation of segregating population of Musa acuminata (AA) for resistance to black and yellow leaf streak diseases.</td>
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<tr>
<td>FHI, Honduras</td>
<td>Tissue culture lab. Shade house Experimental fields Conventional plant pathology lab. ELISA capacity installed PCR capacity (pcoen)</td>
<td>Plant breeder (1) Research assistants (3) Plant pathologists (2) Agronomist (1) Technician (1)</td>
<td>• Breeding</td>
<td>On-going:</td>
<td>M. Rivera, J.F. Aguilar</td>
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<td></td>
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<td>• Field evaluation of new clones</td>
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<td>• Epidemiology</td>
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<td>New proposal:</td>
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<td></td>
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<td>• Population structure of M. fijiensis in Honduras. Project supported by INIBAP/FHI.</td>
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<td>Partners: CATIE (Costa Rica) and CIRAD (France)</td>
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<td>IBP, Cuba</td>
<td>Plant tissue culture lab. Molecular biology lab. Phytopathology lab. Commercial plant tissue culture lab. Greenhouse Shade house Experimental field</td>
<td>Biotechnologist Plant breeders Plant pathologist (2) Molecular biologist Microbiologist Agronomist</td>
<td>• Early screening</td>
<td>On-going:</td>
<td>Y. Alvarado</td>
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<td>• Evaluation of gernplasm in screen-house</td>
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<td>• Genetic transformation (plant/pathogen)</td>
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<td>Perspective:</td>
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<td>• Use of similar test for pathogenesis evaluation</td>
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<td>• Development of methods to identify virulent and avirulent isolates</td>
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<tr>
<td>Plant Pathology Unit,</td>
<td>Pathology lab. Virology lab. Greenhouse Molecular biology and serology facilities</td>
<td></td>
<td>Biological control against fungal diseases</td>
<td>On-going:</td>
<td>P. Lepoivre, J.-P. Busagoro</td>
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<tr>
<td>University of Gemboux,</td>
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<td></td>
<td></td>
<td>• Selection for resistance to plant diseases</td>
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<td>Belgium</td>
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<td>• Study on resistance mechanism</td>
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<td>NRI, University of Greenwic,</td>
<td>Plant pathology and virology laboratories CT rooms and environmental cabinets Electron microscopy Greenhouse Library</td>
<td>Plant pathologist (3) Mycologist (1) Technicians (3) Botanometeorologist (1)</td>
<td>• Epidemiology</td>
<td>Perspective:</td>
<td>P. Burt</td>
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<tr>
<td>UK</td>
<td></td>
<td></td>
<td></td>
<td>• Airborne spread of M. fijiensis.</td>
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<tr>
<td>FABI, University of Pretoria,</td>
<td>Pre-graduate Post-graduate BIOTECHNOLOGY: Microarray facility Sequencer Light cycle MYCOLOGY: Electron and light microscopes Culture maintenance Genetic and molecular analysis of fungi PLANT FACILITIES: Tissue culture laboratories Transformation and GMO facilities Quarantine facilities</td>
<td>Plant Pathologist (2) Mycologist (1)</td>
<td>• Fungal taxonomy</td>
<td>Morphological and molecular identification of Mycosphaerella spp.</td>
<td>A. Viljoen</td>
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<td>of Pretoria, South Africa</td>
<td></td>
<td></td>
<td></td>
<td>• Population genetics</td>
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<td>• Molecular biology</td>
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<td>• Disease management</td>
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<td>New proposal:</td>
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<td>• Morphological and molecular identification of Mycosphaerella spp.</td>
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<td>• Population genetics of Mycosphaerella spp.</td>
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<td>BTI, Cornell University,</td>
<td>Phytopathology labs. Greenhouse &amp; climatic chamber In vitro evaluation facilities Access to in vitro lab. Laboratory of molecular biology (CAPS &amp; microsatellites, sequencers, genomic)</td>
<td></td>
<td>• Molecular genetic tools to study host-pathogen interaction</td>
<td>Molecular genetic tools to study host-pathogen interaction</td>
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<tr>
<td>USA</td>
<td></td>
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<td>Genetic approaches to identify fungal and plant genes expression</td>
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<td>Development of high throughput screening methodologies for evaluating pathogenicity and virulence in plant. Genetic transformation</td>
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<td>Asian institutions, represented by INIBAP Coordinator for Asia represented by INIBAP and Pacific and Executive Secretary of BAPNET</td>
<td>• It has been recommended to take the opportunity of the next BAPNET meeting to have a workshop with Asian participants to the PROMUSA Sigateka working group to define their programme and activities.</td>
<td></td>
<td>• Incidence/severity of Mycosphaerella species on Musa in Asia (morphological)</td>
<td>• Incidence/severity of Mycosphaerella species on Musa in Asia (morphological)</td>
<td></td>
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<td></td>
<td>Evaluation and characterization of various gernplasm collections in Asia against M. musicola, M. fijiensis and M. eumusae.</td>
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<td>Epidemiological studies on M. eumusae.</td>
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<td>Population structures of M. eumusae using molecular tools and corresponding pathogenicity</td>
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<td>Yield loss assessment due to the different Mycosphaerella leaf spot pathogens on Musa.</td>
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</table>
Origin of PROMUSA
As an introduction, Eldad Karamura, INIBAP regional coordinator for eastern and southern Africa, briefed participants on PROMUSA. He explained that PROMUSA was born out of the realization that genetic improvement is the most sustainable strategy for addressing the majority of constraints limiting banana production, particularly for small-scale farmers who account for over 80% of the global production. Consequently, interdisciplinary working groups have been created to generate complementary information needed by the genetic improvement working group. It was clarified that participating in the PROMUSA research agenda did not preclude other research activities on the same topics.

Agenda and outputs of the meeting
The meeting agreed on the following discussion agenda/outputs:

**Airborne dispersal of Mycosphaerella pathogens of Musa - Monitoring of airborne spread of M. fijiensis into uninfect ed areas of the Caribbean**

**Potential partners**
Catherine Abadie - CIRAD
Peter Burt – NRI
Henry Fagan - WIBDECO
Juliane Henderson - CRCTPP
Ronald Vargas – CORBANA

**Development of diagnostic tools for Mycosphaerella species on banana**

**Potential partners**
Jean Carlier and Catherine Abadie - CIRAD
Pedro Crous - CBS
Altus Viljoen - FABI
Juliane Henderson and Elizabeth Aitken - CRCTPP
Kathy Grice and Ron Peterson - QDPI

**Investigation of the durability of the resistance of banana hybrids to M. fijiensis**

**Potential partners**
Catherine Abadie - CIRAD

**Project proposals**
The group also worked on the development of different concept notes to achieve the different recommendations made.

**Determinion of the pathogenic variability of M. fijiensis and M. musicola populations**

**Potential partners**
Yelenis Alvarado - IBP
Ronald Vargas - CORBANA
Laura Conde - CICY
Sergio Aponte - CORPOICA
Zilton Cordeiro - EMBRAPA
Mauricio Guzmán - CORBANA
Galileo Rivas - CATIE

### Name | Institution | Country
--- | --- | ---
Alba S. Riveros | CATIE | Costa Rica
Alice Churchill | BTI, Ithaca | USA
Altus Viljoen | FABI | South Africa
Andres Gutierrez Rojas | CORPOICA | Colombia
Andrew James | CICY | Mexico
Aristoteles Pires de Matos | EMBRAPA | Brazil
Bob Fullerton | Hort Research | New Zealand
Catherine Abadie | CIRAD-FLHOR | France
David Jones | Consultant | UK
Dieter Kaeammer | CICY | Mexico
Elizabeth Aitken | CRCTPP | Australia
Eric Foure | CIRAD/ CARBAP | Cameroon
Fritz Elango | EARTH | Costa Rica
Galileo Rivas | CATIE | Costa Rica
Gus Molina | INIBAP | Philippines
Indra Arlyarathne | ARS | Sri Lanka
Jean Carlier | CIRAD-AMIS | France
Jean-Vincent Escalant | INIBAP | France
Jorge Sandoval | CORBANA | Costa Rica
José G. Garcia Lopez | INIFAP | Mexico
Juliane Henderson | CRCTPP | Australia
Kathy Grice | QDPI | Australia
Laura Conde | CICY | Mexico
Leticia Peraza | CICY | Mexico
Lorna Herradura | BPI | Philippines
Luis Perez Vicente | INVIT | Cuba
Luis Pocasangre | INIBAP-CATIE | Costa Rica
Mauricio Guzman | CORBANA | Costa Rica
Mauricio Rivera | FPHIA | Honduras
Moses Buregyeya | NARO | Uganda
Peter Baint-Kurti | DNA Plant Technologies | USA
Peter Burt | NRI | UK
Philippe Lepoivre | Univ. Gembloux | Belgium
R. Selvarajan | NRCB, Trichy | India
Ron Peterson | QDPI | Australia
Ronald Vargas | CORBANA | Costa Rica
Sergio Aponte | CORPOICA | Colombia
W. Tushemereire | NARO | Uganda
Yelenis Alvarado | IBP, Santa Clara | Cuba
Zilton Cordeiro | EMBRAPA | Brazil

**PROMUSA: Banana weevil working group inauguration**
2 March 2002, Tenerife, Canary Islands, Spain
The following suggestions were made:

- Agree on references/checks.
- Develop screening methods and protocols.
- Identify sources of resistance.
- To compile and exchange information on methods and checks. Standardizing sampling methods is a prerequisite for developing screening methods.
- To have standard protocols for screening germplasm and for identifying sources of resistance.
- To compile information on mechanisms of resistance.
- To assess the possibility of site specific differences regarding resistance.
- To develop research priorities that address the compatibility of genetic improvement with other management practices.
- To consider developing IPM research priorities that contribute to the genetic improvement of the banana.

Certain institutions whose research interests go beyond genetic amelioration, raised the possibility of establishing the working group as a separate entity from PROMUSA for fear of being restricted by the latter’s mission. In the end, everybody agreed there would be a core working group on activities related to genetic improvement, but that the general membership would include policy makers and all those working on banana weevil (including its biology and status as a pest, control methods and technology transfer). It was also suggested to create a list-server to facilitate information exchange on all aspects of banana weevil research.

The fact that PROMUSA focuses on genetic improvement does not mean that other crop protection research activities, e.g. pheromones and entomopathogens, are less important. These should be addressed more efficiently as researchers benefit from the multidisciplinary dynamics created by PROMUSA.

**Way forward**

Formation of the core group – This group should include scientists who actively contribute to genetic improvement, e.g. breeders and scientists working on host plant resistance, mechanisms of resistance, hybrids resistant to weevil, sources of resistance, relevant genetic studies, conventional breeding, biotechnological methods and screening methods.

It was not felt necessary to split this group into scientists working on plantains, bananas or other banana types. For the time being, the group can include anyone working on any aspect of crop improvement of all bananas and plantains.

It was suggested that the group adopt the same procedures for forming this group as those used by other working groups.

It was agreed that:

- Members who were present at this meeting form the working group,
- A convenor should be elected to take charge of the working group,
- The convenor should organize a meeting within the coming year to work out the way forward.

### List of participants

<table>
<thead>
<tr>
<th>Name</th>
<th>Research focus</th>
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</thead>
<tbody>
<tr>
<td>Cliff Gold - IITA</td>
<td>IPM, microbial control, screening, resistance mechanisms, collaboration with breeders.</td>
</tr>
<tr>
<td>Roger Foggai - CARBAP</td>
<td>Integrated Management of the weevil (screening, resistance, biological control).</td>
</tr>
<tr>
<td>Consuelo Castrillon - CORPOICA</td>
<td>IPM, screening.</td>
</tr>
<tr>
<td>Sijin Messeen - KUL</td>
<td>IPM, screening.</td>
</tr>
<tr>
<td>Aurelio Camero - ICIA</td>
<td>IPM, genetic resistance.</td>
</tr>
<tr>
<td>Gloria Labs - ICID</td>
<td>Protease inhibitors, post-harvest evaluation of genetically modified varieties.</td>
</tr>
<tr>
<td>Schalk Schoeman - ARC-ITSC</td>
<td>IPM of weevil, screening for ‘Cavendish’ sub-types.</td>
</tr>
<tr>
<td>Douglas Cubillo - CORBANA</td>
<td>IPM, screening.</td>
</tr>
<tr>
<td>Thierry Lescot – CIRAD-FLHOR</td>
<td>IPM application in diversified systems. Links between research and development.</td>
</tr>
<tr>
<td>Fernando García del Pino – Univ. Autónoma Barcelona</td>
<td>Entomopathogenic nematodes for biological control.</td>
</tr>
<tr>
<td>Angeles Padilla - ICIA</td>
<td>Entomopathogenic nematodes for biological control, artificial diets.</td>
</tr>
<tr>
<td>Dennis Alpizar - Costa Rica</td>
<td>IPM in plantain, pheromones.</td>
</tr>
<tr>
<td>Vincent Ochieng - ICIE</td>
<td>Use of genetics in banana weevil biotyping in relation to control and quarantine.</td>
</tr>
<tr>
<td>Prem Govender - FABI</td>
<td>IPM in commercial banana plantations, active plant pathology group, especially with biotechnology.</td>
</tr>
<tr>
<td>Felix Ortego – CSIC</td>
<td>Activity/ insecticidal protein in insects.</td>
</tr>
<tr>
<td>Miguel Montesdeoca – ICIA</td>
<td>Activity/ insecticidal proteases in banana weevil, pheromones.</td>
</tr>
<tr>
<td>Pedro Castaño – CSIC</td>
<td>Activity/ insecticidal proteins for insect control.</td>
</tr>
<tr>
<td>Caroline Nankinga - NARO/IITA</td>
<td>IPM, entomopathogenic fungi for biological control of weevil, on-farm screening.</td>
</tr>
<tr>
<td>Andrew Kiggundu - NARO</td>
<td>Use of foreign genes for resistance to weevil, protease inhibitors.</td>
</tr>
</tbody>
</table>
EMBRAPA, Brazil, was not represented but may be interested in conventional breeding, biotechnology and screening for local stress. Needs to be contacted.

FHIA, Honduras and EMBRAPA will be contacted to find out their interests (Marline Fancelli).

ITSC, South Africa, proposed to screen new banana varieties, especially ‘Cavendish’ (Schalk Schoeman). The University of Pretoria will supervise students conducting research in banana biotechnology.

CARBAP will screen for resistance to weevils, nematodes and black Sigatoka (Roger Fogan).

IITA has a breeding programme on highland bananas and plantains. IITA works very closely with NARO and banana networks in East and West Africa. IITA is interested in mechanisms of resistance, as well as conventional and biotechnological methods for developing resistance (Cliff Gold).

Election of the convenor

It was proposed that Dr Cliff Gold of IITA be selected as convenor. He has worked extensively on banana weevil, speaks both English and Spanish and has access to communication facilities. He can easily coordinate the preliminary activities. Therefore Dr Cliff Gold was nominated and unanimously approved.

Abstracts of papers presented during the meeting

Session 1. Status of Cosmopolites sordidus in the world

Studies on the banana borer weevil in Cameroon

R. Fogain, S. Messiaen & E. Fouré

CARBAP (Centre africain de recherches sur bananiers et plantains) P.O.Box 832, Douala, Cameroon

In Cameroon, bananas and plantains are a major staple food for a large proportion of the population. A total of 1.7 million tonnes are produced annually. These crops are threatened by a wide range of pests and diseases among which the banana borer weevil (Cosmopolites sordidus) is the major insect pest. For more than six decades, investigations have been carried out on this pest but emphasis was given to testing insecticides that satisfy the needs of large-scale commercial banana plantations. It is only recently that studies on integrated control options were initiated in order to develop control strategies that could also be used by resource limited farmers.

This report presents the activities carried out in Cameroon over the past ten years on the banana borer weevil.

Distribution and population dynamics

Four weevil species are found in the banana and plantain producing areas of Cameroon: Cosmopolites sordidus (Germain), Polythius mellerborgi (Bohemian), Metamasius hemipterus serious (Olivier) and M. hemipterus (L.). C. sordidus seems to be the only weevil of economic importance in banana and plantain plantations (Fogain 1994, Ysenbrandt et al. 2000). The insect is found in all banana and plantain producing areas in Cameroon (Fogain 2001). A survey carried out in all the banana and plantain producing areas showed that the percentage of occurrence of C. sordidus in Cameroon varies between 50 and 90%, and that 82.5% of the farmers are aware of the problem and capable of recognizing weevil damage (Ngamo and Fogain 1998). Research on the population dynamics of the weevil in two of the most important production zones indicates that higher populations are observed between August and September. However, this result need confirmation.

Control methods

In commercial banana plantations, chemical control, the use of clean planting material, and weevil habitat management are the most common methods for controlling weevil populations. Development of alternative control measures and of an integrated pest management strategy are highly recommended for resource limited small-scale farmers, the major producers of plantain.

Chemical control

At the beginning of the 70s, weevil populations were efficiently controlled with Kepone (Chlorodecone) in Cameroon’s commercial banana farms. Between 1975 and 1983, the withdrawal of the product from the market caused a significant increase in weevil populations because of its replacement with HCH and other less effective insecticides, like Dursban (chloropyrifos-ethyl) and Primicide (pyrimiphos-ethyl) (Kehe 1985). The rapid decline of banana production was halted by the arrival on the market of Curlone (Chlorodecone) in the early 90s. With one or two applications per year, weevil populations where effectively controlled. But the product was soon withdrawn from the market, unfortunately with nematicides, like Counter (terbuthyl) and Furadan (carbafuran), that have insecticidal activity and can be used when populations are relatively low. In the Mungo department, the threshold for treatment in industrial banana plantations is when 5% of the 20 sampled mats per hectare are attacked, based on the method proposed by Vilardebo (1973). Other insecticides with interesting properties are: tebupirimphos, athiamethoxam, cartap and imidacloprid.

Timely chemical control is an efficient way of knocking down adult weevil populations in commercial farms, but is too expensive for the majority of resource limited farmers and has unfavourable side effects on beneficial non-target organisms. According to a survey conducted in southwest Cameroon, 57% of smallholder farmers said they did not use pesticides (Chantelot 1993). Forty-three percent, mostly in mixed plantain-cocoa plantations, treated suckers before planting and 87% used insecticides generally referred to as ‘gabarine’, insecticides used against timber or cocoa pests and which include lindane (HCH), Dursban (chloropyrifos-ethyl) and methylparathion. Three percent of the farmers who treat suckers before planting use a nematicide with insecticidal activity, such as Mocap (Ethoprophos), and 10% use other products (Chantelot 1993). Another survey in west, southwest, central and south Cameroon revealed that only 11% of smallholders use pesticides, 57% do not use anything and more than 32% use ashes because they believe it controls weevils (Ngamo and Fogain 1998).

Cultural control

It is important to plant an uninfested field with clean planting material which can be obtained...
from weevil-free plantations or tissue culture facilities. Ninety-five percent of smallholders practice paring of suckers before planting (Chantelot 1993), but since the availability of good planting material is a major limitation in Cameroon, infested suckers of minor quality are often planted.

Residual corms in the soil should be destroyed and post harvest residues slashed in order to prevent the multiplication of weevils. Weeding should be done regularly in order to avoid development of a favourable humid weevil habitat. In smallholder farms, habitat management is neglected because labour is limited or rented labour is not productive enough. Weeding is minimal (two to three times a year) and herbicide application is rare. A minority of farmers prop with bamboo even though the practice can give good results with minimal investments. In a survey carried out between February 1997 and March 1998 of 240 plantain plants in eight smallholder farms, plant losses due to nematodes, weevils, and water and nutrient stress represented 6% of the losses, of which 37% were due to toppling or falldown (mainly at the beginning of the rainy season, due to violent winds) and 29% were due to breakage of the pseudostem (mainly at the end of the dry season due to water stress) (Anonymous 1998).

More than 30% of smallholder farmers use household ashes at planting because they believe it reduces damage to the corm (Ngamo and Fogain 1998). It is not clear whether ashes have an insecticidal or merely a fertilizer effect. Under laboratory conditions, ashes have a repellent effect on adult C. sordidus, but the toxicity to adults is quite low (Messiaen 1999).

Commercial banana plantations are renewed every five to six years. During the fallow period, residual corms are usually destroyed by local women who use the fallow field for food crop production. Tissue cultured plants are treated with Regent5G (fipronil) or Counter10G (terbufos) at planting and two or three times a year. Crop hygiene (weeding, herbicide application, slashing of residual pseudostems and topped mats) as well as propping and guying are commonly practised. Biological control

At CARBAP, research on biological control using the entomopathogenic fungus Beauveria bassiana started in 1994 with the discovery of local strains in Cameroon (Fogain 1994). Since then, studies have been carried out under controlled conditions to test the efficiency of the strains and the possibility of mass production for field trials. Three strains of Beauveria bassiana, isolated from infected weevils caused 92% mortality after nine days under laboratory conditions. Research is presently carried out on the maintenance of viability with regards to delivery systems and the feasibility of mass production for farmers or economic agents in Cameroon. Entomopathogenous nematodes have been isolated from soil samples collected in Cameroon using C. sordidus larvae.

Use of botanicals

Dipping suckers in a 20% neem (Azadirachta indica) seed solution at planting protects the young suckers from weevil attack for several months, but a crown application three times a year is not effective in reducing damage (Fogain and Ysenbrandt, 1998). It achieves this result by reducing oviposition, through its repellent effect on adult weevils, and by blocking egg hatching (Messiaen 1999).

Trapping

Trapping using pseudostem traps does not always reduce weevil populations, depending on the cropping system, the level of weevil immigration from neighbouring infested plots, the number of traps placed and the initial population. In Cameroon, trapping does not seem to be a viable control option in smallholder farms because of the unrealistic amount of pseudostems and labour needed, and because of weevil immigration from adjacent plots.

Testing of a mass trapping system using ramp traps baited with sordidin, an aggregation pheromone, indicated that the traps were not attractive enough to constitute a viable control option in industrial banana plantations, but additional research is needed to assess whether the attractiveness can be improved with another type of trap and kairomones. In smallholder farms, pheromone mass trapping does not seem to be a viable option for controlling weevil populations because of problems of storage and costs (Messiaen 2000a).

Host plant resistance

Screening for resistance to the banana borer weevil at CARBAP stated in the 1994 with the discovery of the field resistant ‘Yangambi km5’ and of the highly susceptible clones of the plantain subgroup (Musa AAA) compared to the ‘Cavendish’ (Musa AAA) (Fogain and Price 1994). Since then, techniques for early screening in the field and under controlled conditions have been refined. In a recent screening, more than 80 varieties were tested. Several varieties, including CARBAP hybrids, have been selected for enhanced screening in the field. Results of preliminary screening show a large variety of responses to weevil attack between and within genomic subgroups (Messiaen 2000b). No genotypes are more susceptible to weevil attack than the ones in the plantain subgroup. Preliminary results point to differences in larval development. If the results are confirmed in the field, it will be possible in the short or medium term to develop hybrids partially resistant to C. sordidus.

Conclusions

Significant information has been gathered over the past ten years on the distribution and population dynamics of the weevil. Despite gains in knowledge on the dynamics of C. sordidus in the Fako and Mungo divisions (littoral and southwest Cameroon), investigations are needed for other provinces, such as the major plantain producing areas of the centre and south (Anonymous 2000). The insect was found to be present everywhere in the country where bananas and plantains are produced. A larger spectrum of insecticides is available, but mainly to commercial growers. Several insecticides from different chemical groups are now available and can therefore be used in rotation to avoid the development of resistance to C. sordidus. As for biological control, other nematomes, neem (A. indica) and the entomopathogenic fungus B. bassiana show great potential for weevil control. But field trials are needed to confirm greenhouse results. Sources of resistance to the weevil have been identified and breeding programmes can now use them to develop genotypes resistant to the insect.

Research on other non-chemical control methods, such as cultural control and the use of pheromones, continues.
The biology and management of the banana weevil, Cosmopolites sordidus in South Africa

P. Govender1 and A. Viljoen2

1 Department of Zoology & Entomology; 2 Department of Microbiology & Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

The banana weevil, Cosmopolites sordidus, introduced in South Africa about 30 years ago, is the most important insect pest of banana, causing economic losses in the Pumalanga and the south coast of KwaZulu-Natal regions. Collectively this area represents about 78% of the total 12 078 hectares under commercial banana production in the subtropical pockets of South Africa. The weevil has a limited potential to migrate from its current distribution area unless it is transferred with infected planting material. Information on the life cycle appears to be consistent with published literature; the total developmental period being about 33 days. Adults emerge during spring and late summer, and their nocturnal activity increases during or after rainfall. Females generally lay one egg per week from late August to February but this number can increase during optimal environmental conditions and low pest densities. Adults have a life span of about two years. Although weevil numbers are low in the winter months (May to July), they increase rapidly in spring and early summer (August to November). Weevils are monitored using pseudostem traps at a density of 50 traps/ha. Economic threshold values are 1–2 adults/trap/week and 10 or more larval nocturnal activities equally to plant loss and bunch weight reduction. By the 7th cycle of the second trial, 35% of the mats had died out in weevil-infested plots compared to 2% in controls. Overall yield losses were 50% for the trial.

Although pesticides can be an effective control method, in Uganda the weevil has developed resistance to one chemical. IITA and the Ugandan National Banana Research Programme work closely together on cultural and biological controls, and on host plant resistance. Clean planting material is an important means of keeping weevils out of new plantations, but the effect normally disappears within a few crop cycles. A one-year trapping study showed some positive effects on population reduction but this control is beyond the resources of most Ugandan growers. Current emphasis is on the use of neem, endemic ants, microbial control (i.e. Beauveria bassiana and endophytes) and host plant resistance. Available data indicate that all highland banana clones are susceptible to weevil. However screening trials suggest that many resistant Musa clones do exist and that antibiosis is the predominant means of resistance in these clones.

Cosmopolites sordidus in the autonomous region of Madeira

Luís Nuno and V. P. Ribeiro

Direcção Regional de Agricultura da Regional Autónoma da Madeira – Portugal

The agricultural service of the government of Tenerife Island (Cabildo) did a series of surveys to determine the extent and severity of damages caused by Cosmopolites sordidus Germar. Carried out between 1996 and 2001, the surveys were repeated every three years, depending on the study area. Peeled off pseu
dostem double-disc traps and those with a horizontal section of the corm were highly correlated (R2=0.93). The distribution of C. sordidus in relation to the type of irrigation, the altitude of the plantation and the variety used (‘Pequeña enana’ or ‘Gran enana’) was also studied. Time trends were done to see the evolution in the distribution of this pest and the damages it causes.

Management of banana and plantain weevil borer in Costa Rica

Douglas Cubillo y Mauricio Guzmán

Sección de Fitopatología y Entomología, Dirección de Investigaciones de CORBANA S.A., Costa Rica

Chemical control and cultural practices are the base for the management of banana weevil in plantain and banana plantations in Costa Rica. Usually the damage of the pest is more important in plantains (Musa AAB) than in bananas (Musa AAA), due to differences in...
Session 2. Control of the banana borer weevil

**Overview of Beauveria bassiana for microbial control of the banana weevil in Uganda**

C.M. Nankinga¹, C.S. Gold¹, W. Tushemereire²

¹International Institute of Tropical Agriculture, Eastern and Southern Africa Regional Centre, P.O. Box 7878, Kampala, Uganda; ²National Banana Research Programme, Kawaanda Agricultural Research Institute, P.O. Box 7065, Kampala Uganda

Collaborative research is being conducted in Uganda to assess the microbial control potential of *Beauveria bassiana* (Balsamo) Vuillème (Hyphomycetes) for the banana weevil, *Cosmopolites sordidus* (Germar), (Coleoptera: Curculionidae). Since the early 1990s, isolation, characterization and pathogenicity studies have come up with a selection of indigenous isolates of *B. bassiana* that have good growth and production traits, causing 50-100% weevil mortality within 10-21 days after inoculation, depending on the isolate. In small-scale field trials conducted at Kawanda Agricultural Research Institute, one *B. bassiana* isolate (code G41), which showed high pathogenicity to *C. sordidus* as well as superior growth and sporulation compared to other isolates, was tested. Three methods of delivering *B. bassiana*, namely (i) application of the fungus on topsoil around the base of the banana mat (ii) application of the fungus with pseudostem and disc-on stump traps and (iii) application of the fungus to banana planting suckers, were evaluated. Treating banana suckers with a *B. bassiana* dry maize culture formulation and a soil-maize based formulation (2.3 x 10¹² conidia/planting hole), reduced weevil damage by 20-30% within a period of eight weeks after planting in holes dug in a 2 to 3-year-old banana field of local EAAH cooking cultivar. Dead *C. sordidus* adults and larvae with *B. bassiana* fungal growth were observed in the treated suckers indicating immature stage infection. When the maize and soil-based formulations of *B. bassiana* were applied beneath the pseudostem and disc-on stump traps, it was observed that the moist conditions under the traps, in addition to attracting weevils, also provided a favourable environment for extra sporulation of *B. bassiana* and this enabled the fungus to remain potentially infective. *B. bassiana* cultures collected from the field traps in the first five weeks after application were highly infective causing 60-100% weevil mortality in 14 days, but the infectivity of the fungus was significantly reduced in the wet season; likely due to contamination by other soil micro-organisms. The maize culture formulation (2 x 10¹⁰ conidial/ha) and maize-soil based formulation (2 x 10¹⁰ conidia/ha) applied at the base of the mats reduced the adult banana weevil populations by 30-50% and kept them at lower levels than in the untreated plots. The treated plant also showed reduced weevil damage and up to 16% *B. bassiana* disease infection was observed in dead weevils in the field.

Previous studies have demonstrated that good potential exists for the use of *B. bassiana* for microbial control of the banana weevil. The collaborative team, composed of scientists from the International Institute of Tropical Agriculture (IITA), the National Banana Research Programme of the Uganda National Agricultural Research Organization (NARO), CABI Biosciences UK and University of Reading is undertaking further research into the mass production and formulation of *B. bassiana* and exploring other delivery systems of *B. bassiana* for integration with other control measures under farmers’ conditions. Research is geared to developing economically viable mass production, formulation and delivery systems that will overcome the problems associated with field fungal efficiency, persistence and transmission. Further research on the ecological relationships between banana weevils and entomopathogens will also be undertaken to understand the conditions under which *B. bassiana* is likely to be most effective in controlling this pest. This include studies on the behaviour of the weevil, which might influence the likelihood of the insect contacting the pathogen; the bio-types in the *Cosmopolites sordidus* species, which might exhibit different susceptible levels to the pathogen; and pathogen viability and virulence under aerobic (ordinarily pseudostem traps) and anaerobic conditions such as in the semi-chemical-based trapping systems. We acknowledge funding from the Rockefeller Foundation, DFID and BMZ in support of this work.

**Entomopathogenic nematodes for the control of insect pests. The outlook for the control of Cosmopolites sordidus**

Fernando García del Pino
Universidad Autónoma, Barcelona, Spain

Entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) are used in biocontrol against different insect pests in soil and cryptic habitats. They are symbiotically associated with bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively. Nematode dauer juveniles, harbouring cells of their specific bacteria in their intestine, search for insects in the soil. After penetration in the host insect, they release their symbionts. The bacteria multiply and produce suitable conditions for nematode reproduction in the dead insect. After about two weeks, dauer juveniles emigrate from the cadaver and search for a new host.

The use of entomopathogenic nematodes for the control of *Cosmopolites sordidus* should now be economically feasible. Production and formulation techniques have been improved to provide these nematodes to growers at a cost equivalent to or lower than the one of chemical insecticides. Application of entomopathogenic nematodes requires less labour than insecticides, avoids the problems of insecticide resistance and has little or no adverse effects on the environment. However, for reliable results, it is necessary to improve the application technique, and to select the appropriate species and strains of entomopathogenic nematodes. Finally, various strategies for the control of *Cosmopolites sordidus* using entomopathogenic nematodes are discussed.

**The use of two insecticide-nematicides to control the weevil *Cosmopolites sordidus* and the nematode *Radopholus similis* and their effect on some production variables of the banana ‘Gran enana’ in relation to the environmental conditions encountered in Costa Rica**

Dennis Alpízar M.

In Costa Rica, insecticide-nematicides are commonly used to control *Cosmopolites sordidus*. Pseudostem or corn traps are also used while the utilisation of the aggregation pheromone Cosmolure®, introduced at the end of the 90s to control the banana weevil, is still a new agicultural practice in the country’s banana and plantain plantations.

The objective of this study is to compare the effect of using or not using, under the same conditions over two years, two insecticide-nematicides: terbufos (four applications) and etopros (one application). After two years the results show, using the Fisher t-test (0.05), that the ‘weight of the bunch’ was slightly but significantly higher in the non-treated plots while the ‘number of nematodes (Radopholus similis) in the functional root of the sucker’ was slightly lower in the treated plots. The other variables were not statistically different.
The cost of applying the insecticide-nematicide was SUS 1200 per hectare for the two-year duration of the study.

Alternatives to the control of Cosmopolites sordidus (German) in banana plantain plantations

A. Padilla Cubas, F. García del Pino, L.V. López Llorca and A. Camero Hernández

In the Canary Islands, Cosmopolites sordidus (German) is the most important pest in plantations of banana plantain. Given the results of chemical treatments, alternatives are sought to control the weevil. We therefore sampled soils, cultivated and not cultivated, in the province of Santa Cruz de Tenerife looking for parasitized organisms. Using ‘Galleria mellonella’ traps, we especially searched for entomopathogenic nematodes and fungi.

Entomopathogenic nematodes were found in two sampling points from which we isolated Heterorhabditis spp. and Steinernema spp. As for entomopathogenic fungi, we isolated Aspergillus flavus, Beauveria bassiana, Metarhizium anisopliae and Paecilomyces spp. Verticillium lecanii was isolated from white flies collected in different localities.

We characterized the morphology of the fungi and studied their germination and sporulation capacity, their production of biomass and their behaviour under various naturally occurring conditions of humidity, temperature and pH. We also studied their enzymatic activity: quiti-no, amylo, proteo, lipo and pectinolytic.

We also conducted biological assays using ‘Galleria mellonella’ and, based on the results, we inoculated C. sordidus using two methods. Finally, we evaluated the interactions between these isolates and Fusarium oxysporum, the main pest of banana.

Behaviour of entomopathogenic fungi on vegetal tissue

L.V. López Llorca

We studied the use of ornamental plant vegetal residues from nurseries to produce inocula of antagonistic fungi, including entomopathogenic fungi.

Seeds of Phoenix dactylifera turned out to be excellent for the production of Beauveria bassiana. Scanning electron microscopy revealed a very porous substrate, a feature which facilitates fungal development and sporulation. A soil-based formulation of B. bassiana can sporulate and overcome fungistasis. Thanks to this type of seed, the fungus can survive and maintain itself in the soil during at least three months. During bioessays, the mixture infected a pest of palm trees (Carpophilus dimidius) similar to the weevil. Moreover, B. bassiana can colonize the petioles of P. dactylifera. We think that such endophytic behaviour is useful in controlling pests like the weevil.

Research on the Musa borer weevil (Cosmopolites sordidus) at CARBAP

Eric Fourné, S. Messiaen, Roger Fogain, Thierry Lescoat

CARBAP, B.P. 832, Douala, Cameroon; *CIRAD-FLHOR BPA, Boulevard de la Lironde, T504/P54, 34 398 Montpellier Cedex 5, France

The banana borer weevil Cosmopolites sordidus is the most important pest of banana and plantain in African plantations. The African Center of Banana and Plantain (CARBAP) carries out research on C. sordidus, with a focus on integrated pest management, namely:
• genetic resistance,
• population dynamics,
• biological (bio-insecticides and pheromones) and chemical control.

Results are presented on selection for weevil resistance; weevil evolution; effects on plantain production in southwest Cameroon; best parameters for field infestation representation; efficiency and limits of chemical insecticides; use of pheromones in trapping system; efficiency of Beauveria bassiana in southwest Cameroon; and use of insecticide plants against weevils.

Research on the Musa borer weevil (Cosmopolites sordidus) at CIRAD

Christian Chabrier, Thierry Lescoat

CIRAD-FLHOR BPA, B.P. 153, 97202 Fort de France, Martinique, France; *see above

The Banana, Plantain and Pineapple programme (BPA) of CIRAD-FLHOR carries out research in the French West Indies and in the central laboratories of Montpellier, France, as well as collaborations with CARBAP in Cameroon and applications in Indian Ocean islands.

The research focuses on integrated pest management along the same lines as CARBAP.

The results presented focus on the combination of two types of synthetic pheromones; the use of entomopathogenic bacteria and nematodes; and the efficiency and limits of chemical insecticides.

Session 3. Molecular biology

Resistance of diploid banana genotypes to Cosmopolites sordidus (Germ. 1824) (coleoptera: curculionidae)

M. Fancelli, A. Souza Do Nascimento, N. Fritzons Sanches, R. Correa Caldas and S. De Oliveira E Silva

EMBRAPA, Mandioca y fruticultura, Rua EMBRAPA s/n, Caixa Postal 007, 44380-000 Cruz das Almas, Bahia, Brazil

Plant resistance to insects is considered a secure and durable strategy for the control of Cosmopolites sordidus, especially in plantations where investments are low. Despite the existence of a large number of varieties, the number of cultivars used in Brazil is small, hence the importance of evaluating the new genotypes introduced and/or generated in genetic improvement programmes. Although in the fields all varieties are infested, some studies show differences among genotypes with respect to development, survival and attractiveness for oviposition. Given the current expansion of banana plantations in Brazil and the development of methodologies for the production of in vitro plantlets, there is a growing interest for improved varieties, including resistant ones. The tetraploid hybrids of banana (AAAB) are obtained by crossing diploid (AA) genotypes with triploid (AAB) cultivars of types Prata (Silver) and Maçã (Pomme). A genetic improvement programme of diploid genotypes is carried out, with the aim of increasing yield and resistance to pests, one more reason for a close partnership between breeders and geneticists.

The objectives of the present work are:
• To evaluate diploid hybrids of banana in relation to Cosmopolites sordidus.
• To study the mechanisms of resistance to the weevil in diploid genotypes.

Methodology

The following genotypes are being studied: 0304-02; 0337-02; 0323-03; 1318-01; 2803-01; 4223-03; 5012-02; 4215-02; 4279-13 and 4252-03. These materials are diploid hybrids generated by the Banana Genetic Improvement Programme, most of them presenting resistance to black Sigatoka. Plantlets from these genotypes were placed in screened planting holes in the field and infested with adults of the banana weevil using the methodology adopted by Seshu-Reddy and Lubega (1983). Plants without the insect are kept under the same conditions to get information on the injuries caused by the pest. The genotype Terra is being used as susceptibility control.

The variables analyzed are: coefficient of infestation, number of insects present in the galleries, plant height, pseudostem diameter, time period until inflorescence emission, yield, bunch weight, number of hands,
finger diameter and number of fingers/hand. Under laboratory conditions the development of the insect and its non-preference for feeding and oviposition will be studied for the same genotypes in order to identify the types of resistance involved in the interaction between the weevil and the banana plant. The duration and viability of the larval and pupa phases, the weight of the pupa after 24 hours and the number of adults with defects, will be noted. Tests for attractiveness and consumption will be carried out. Analyzes will be done to identify the presence of attractive/repellent substances, as well as phago stimulants and/or phago deterrents. Rhizome hardness will be evaluated using a penetrometer.

Reference

Aspects of banana weevil resistance in Musa and prospects for genetic engineering against the banana weevil

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1National Banana Research Programme, Kawanda Agricultural Research Institute, P.O. Box 7065, Kampala, Uganda and the Forestry and Agricultural Biotechnology Institute, University of Pretoria, 74 Lunnion Road, Hillcrest, Pretoria, 0002 South Africa; 2International Institute of Tropical Agriculture-East and Southern Regional Center (IITA-ESARC), in Uganda, we found that East African highland bananas (EAHB) (AAA-EA) and plantains (AAB) were the most susceptible. These were followed by ABB bananas (cv ‘Pisang awak’ and ‘Bluggoe’), diploid banana derived hybrids, AB bananas (cvs ‘Ndizi’ and ‘Kisubi’), AAA bananas (cvs ‘Yangambi km5’, ‘Cavendish’ and ‘Gros Michel’) and the wild AA type ‘Calcatta 4’ being the most resistant. Several factors were shown to contribute to weevil resistance. Dry matter content (representing corm hardness), resin/sap production of the corm and sucking ability were important for weevil resistance in all accessions. Corm diameter (size) was also important in the EAHBs. Preliminary investigations into the chemical basis of resistance, using high-performance liquid chromatography profiles of corm extracts, indicated that compounds present in some resistant cultivars (especially those with a genome) negatively correlated with weevil damage. These compounds were absent in most of the susceptible cultivars tested.

Ortiz et al. (1995) studied the genetic inheritance of banana weevil resistance and found it to be under the control of more than one gene with partial dominance towards susceptibility. They found significant additive effects and modifier genes plus dosage effects of susceptibility genes, causing higher susceptibility at higher ploidy levels. Conventional crossbreeding in combination with molecular biotechnology techniques, like marker assisted selection (MAS) and genetic transformation, appears to be an attractive option to further understand resistance to weevil and simultaneously develop resistant cultivars. Sources of transgenes can include the Musa genome itself and others of plant or animal origin (Carozzi and Koziel 1997). The beauty of genetic engineering is that genes from several sources can be exploited and that these can be transformed using a gene pyramiding strategy. However, a great deal of information about the complex nature of weevil resistance is missing and molecular marker analysis can assist in genetic analysis and mapping. Opportunities also exist for quickly developing weevil resistance through genetic transformation.

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Genetic biodiversity in banana weevil (Cosmopolites sordidus) from different banana growing regions

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Genetic variability in banana weevil populations was analyzed in a large number of samples obtained from 15 banana growing tropical countries using random amplified
Alternatives to control the banana borer weevil, Cosmopolites sordidus Germar (Coleoptera: Curculionidae), on banana

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The banana borer weevil, Cosmopolites sordidus Germar, is considered the main pest of banana in the world. In the Canary Islands, it was first noticed in 1945 and eradicated. It reappeared in 1987, but despite attempts to control it by chemical and cultural means, the pest has spread causing increasingly severe damages.

A project (RTA 02-100-C3) was recently initiated to develop an integrated pest management strategy against the weevil. Its aim is to maintain the profitability of the culture by producing an environmentally friendly banana that should have a competitive edge on European markets over bananas from other producing countries.

The objectives of the project are: 1) to optimize the use of pheromone to control the weevil; 2) to evaluate the relation between the extent of the damages caused by the weevil and yield losses; 3) to determine weevil inter and intra-population variability in relation to biological control; 4) to identify and study the behaviour of the entomophagous organisms attacking the weevil.

Our participation in this project consists in determining weevil inter and intra-population variability among the populations affecting the banana plantations of Tenerife, Gomera and La Palma, in order to provide information which could help in controlling this pest, such as the origin and the dispersal patterns of these weevils. Detecting DNA polymorphism will be done using the randomly amplified polymorphic DNA technique, following the methodology used to study the population genetic of another species of Curculionidae, a pest of sugarbeet in Andalusia (Taberner et al. 1997, J. Mol. Evol. 45:24-31).

We also plan to identify and characterize the digestive enzymes of the weevil, and to conduct bioassays with protease inhibitors in order to determine their effect on the survival and development of this pest. This information is necessary for the eventual production of transgenic banana plants expressing defence proteins, a possibility which would offer new means of controlling the weevil. Preliminary studies suggest that larvae and adults possess a complex proteolytic system which includes aspartyl, cysteine and serine endoproteases as well as amino and carboxypeptidases. Once the characterization of digestive proteases is finished, we will study their interactions with specific inhibitors in order to determine which inhibitors, or combination of inhibitors, could be incorporated by genetic manipulation in potentially resistant banana plants.

Fourth and final FAO/IAEA research coordination meeting on Cellular biology and biotechnology including mutation techniques for creation of new useful banana genotypes

Summary report
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Bananas and plantains are grown in over 100 countries throughout the world with annual production around 88 million metric tons. Banana fruit production is severely limited by several diseases and pests such as banana bunchy top virus, burrowing nematodes (Radopholus similis), Moko disease (Ralstonia solanacearum), black Sigatoka or black leaf streak (Mycosphaerella fijiensis), Fusarium wilt (Fusarium oxysporum f.sp. cubense). FAO/IAEA started a banana Coordinated Research Project (CRP) in 1994 with the general aim to integrate radiation induced mutations in vitro culture and molecular genetics methods into the conventional breeding of banana to induce desirable variation such as disease resistance, dwarfism and earliness, and also to promote the development of methods for large-scale and rapid multiplication of the mutants/segregants through somatic embryogenesis and micropropagation. The Belgium Government decided to fund this CRP in 1996. Since then, three Research Coordinated Meetings (RCM) were held in different countries including Vienna, Malaysia and Sri Lanka. The fourth and final RCM of this banana CRP was held at the Katholieke Universiteit Leuven (KULeuven), Leuven, Belgium, 24-28 September 2001. A total of ten participants attended it including from Austria (FAO/IAEA), Belgium, Cuba, Czech Republic, Germany, Israel, Mexico, Philippines, and Sri Lanka. The results of CRP will be published in a book entitled “Banana improvement: Cellular and molecular biology, and induced mutations”.

Overall achievements
Research tools were developed for germplasm characterization and improvement through induced mutations, cryopreservation, somatic embryogenesis, somaclonal variation and genetic engineering. Some of the existing cultivars have been improved for disease tolerance and important agronomic traits. Collaborations among participating laboratories were established, including exchange of staff, training and technology transfer.

Practical achievements
Research contract holders J. Lopez Torres (Cuba), Mak Chai (Malaysia), A. James (Mexico), and J. Dolezel (Czech Republic) did exceedingly well in this CRP. Nicolas Roux (FAO/IAEA) has been instrumental in disso-
transformation rate was cultivar dependent. Were used for banana transformation, and information and particle bombardment methods 4. Both different traits such as early flowering, reduced improved clones that were screened for dif- for cryopreservation of banana was pub- published at the International Institute for banana viruses was organized. Post-graduate training on indexing of beauty viruses was organized. Flow cytometry facilities were estab- lished at the International Institute for Tropical Agriculture (IITA, Nigeria) and The Malaysian Institute for Nuclear Technology MINT, Malaysia). The transfer involved staff training in the Institute of Experimental Botany (IEB, Czech Republic) and expert visit.

Specific achievements 1. Detection of DNA methylation polymorphism in banana micropropagated plants with amplified fragment length polymorphism (AFLP). 2. Somatic embryogenic cell suspension cul- tures (ECS) were developed for several banana cultivars including plantains (AAB). Three cryopreservation techniques were developed for long-term conservation of meristems. An INIBAP technical guideline for cryopreservation of banana was pub- lished in English, French and Spanish. 3. Induced mutations generated a series of improved clones that were screened for dif- ferent traits such as early flowering, reduced height, large fruit size, and tolerance to Fusarium. 4. Both Agrobacterium-mediated transfor- mation and particle bombardment methods were used for banana transformation, and transformation rate was cultivar dependent.

5. Virus indexing procedures were trans- ferred to Sri Lanka for indexing local banana virus strains. 6. An early screening technique was devel- oped for Fusarium wilt using tissue culture- derived plants in a double-tray system. 7. A selection system was developed against black Sigatoka disease by using Mycosphaerella fijiensis crude extract, the semi-purified, and one purified fraction (juglone). 8. Screening techniques for nematode resistance were developed in Musa under shade-house and field conditions. Aseptic cultures of Radopholus similis and Pratylenchus coffeae were established using alfalfa calli, and their pathogenicity was confirmed after greenhouse tests. 9. DNA flow cytometry was used for detection of polyploidy, monitoring of cytochima dissociation, and analysis of karyological stability of ECS. 10. Transposon mutagenesis was explored for gene tagging, using maize Ac element, in banana genome. A substantial number of distinct mutants were generated and characterized.

11. Fluorescence in situ hybridization (FISH) protocol was developed for Musa for detailed studying of karyotypes, providing distinct chromosome landmarks, gene local- ization, analysis of long-range chromosome structure, and linking to physical and genetic maps. 12. A total of 28 allele-specific simple sequence repeat (SSR) markers were gen- erated for Musa and used to detect: poly- morphisms between the A and B genomes, identify hybrids, and trace back the B genome in hybrids. These markers are now used within the CRP and worldwide. A total of 24 locus-specific, highly polymorphic SSR markers were also produced for Mycosphaerella fijiensis to discriminate them from other species.

Abstracts of papers presented during the 4th and final FAO/IAEA research coordination meeting

Detection of DNA methylation changes in micropropagated banana plants (Musa AAA cv. ‘Grande naine’) using the methylation-sensitive amplification polymorphism (MSAP) technique

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The extent of DNA methylation polymorphisms was evaluated in leaf tissue of micropropagated banana plants (Musa AAA cv. ‘Grande naine’) derived either from the vegetative apex of the sucker or the floral apex of the male inflorescence using the methylation-sensitive amplification polymorphism (MSAP) technique, which utilizes the restriction isochizomer pair Msp I and Hpa II, whose ability to cleave at the sequence 5’-CCGG-3’ is affected by the methylation state of the cytosines. In all, 465 fragments, each repre- senting a recognition site cleaved by either or both of the isochizomers were amplified using eight combinations of primers. A total of 107 sites (23%) were found to be methylat- ed at cytosine in the genome of micropropa- gated plants. The highest number of DNA methylation polymorphisms was detected in plants micropropagated from the male inflor- escence with 14 (3%) and the lowest in plants micropropagated from the sucker with 8 (1.7%). These differences were not statisti- cally significant. In leaf tissue of convention- ally propagated plants DNA methylation poly- morphisms were not detected. Micropropagated plants were relatively hyper- methylated in comparison to conventionally propagated plants, with some bands being methylated in all micropropagated plants but non-methylated in all conventionally propagat- ed plants. These results demonstrated the usefulness of MSAP to detect DNA methyla- tion events in micropropagated banana plants and indicate that DNA methylation changes are associated with micropropagation.

Discovery of functional genes in the Musa genome

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Development of stable and reproducible transformation and regeneration technologies opened new horizons in banana and plantain breeding. Several transformation strategies have been published in the last five years by
different banana biotechnologists. Disease resistance and improvement of fruit quality have been the focal points of most Musa breeders. However, despite growing interest in banana biotechnology, the pool of Musa genes in public databases is relatively small (of the approximately 300 accessions placed in the NCBI database less than 25% are annotated cDNA’s). Our laboratory is currently employing several approaches for the identification of functional genes in the Musa genome. These include transposon tagging, high throughput random mutagenesis, suppression subtractive hybridization (SSH) and bioinformatic analysis of clustered ESTs.

We have introduced the maize Ac transposable element into the Musa genome and followed excision and insertion of the element in numerous transgenic lines. The goal was to investigate the frequency of transposition and distribution of insertions along chromosomes. The constructs we have used include an Ac element fused to GUS reporter under the 3SS promoter. PCR analysis of a variety of mutants revealed that most carried a chimeric pattern with regard to expression of the foreign genes. Consequently, only a few transgenic lines (tissue cultured siblings) showed detectable differences in the banding pattern on Southern hybridization blots. Attempts were made to stabilize the Ac element following a limited number of transpositions, by silencing the gene encoding the transposase enzyme after excision.

Differentially expressed genes, which are activated in the post climacteric phase of fruit development, were analyzed in the peel and pulp of banana fruit. Using suppression subtractive hybridization (SSH) we have isolated over 200 partial cDNA’s encoding genes which are expressed during the final stages of fruit development (senescence). High throughput screening by membrane hybridization was employed for preliminary selection of candidate genes involved in regulation of the onset of senescence. Sequence analysis and blasts against GeneBank databases revealed approximately eighty non-redundant clones, which were up regulated in the post-climacteric phase. Most, but not all of these genes were up regulated, after exposure of green fruit to 1000 ppm ethylene for 24 hours. The sequenced pool of up-regulated cDNA’s fall into one of three major categories:

Genes involved in metabolic processes, mainly carbohydrates and lipid components.

Genes involved in cellular regulation (protein kinases, transcription factors etc.).

Genes involved in protection from pathogens and environmental stress conditions - metallothionein like protein, super oxide dismutase, osmotin-like protein, pathogen related proteins etc.

A significant number of sequences showed no substantial homology to functional genes in the GeneBank.

**Analysis of Musa genome using flow cytometry and molecular cytogenetics**

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This project focuses on the analysis of Musa genome at nuclear and chromosomal level with the aim to understand long-range organization of Musa chromosomes and to characterize changes of chromosome structure during speciation and evolution of cultivated clones.

We have used flow cytometry to determine ploidy levels of Musa accessions held at the INIBAP Transit Center (K.U. Leuven). Flow cytometric ploidy assay involved preparation of suspensions of intact nuclei from small amounts of leaf tissue and the analysis of fluorescence intensity after staining with DAPI. Chicken red blood cell (CRBC) nuclei were included in every sample as an internal reference standard (Figure 1). From the 890 accessions analyzed so far, 8.4% were classified for the first time, and 7.6% accessions exhibited other ploidy then reported previously. In 2% of the accessions, plants of mixed ploidy were detected. A reliable and high-throughput system for ploidy screening in Musa is an important outcome of the study. The use of CRBC nuclei, allowed high-resolution analysis, and the results obtained so far indicated suitability of this system for rapid detection of aneuploidy. As the materials for analysis were sent by express mail, this work demonstrates that it is possible to perform flow cytometric ploidy analysis in distant laboratories.

In attempt to characterize DNA sequences contributing to structure and evolution of Musa chromosomes, we have constructed partial genomic DNA libraries in *M. acuminata* and *M. balbisiana* and screened them for clones carrying highly repeated sequences, and sequences carrying rDNA. Isolated clones were characterized in terms of copy number, genomic distribution in *M. acuminata* and *M. balbisiana*, and sequence similarity to known DNA sequences (Table 1). In contrast to many plant species where mobile elements and their remnants contribute most of the nucleotide content, our observations indicate that these elements do not represent a major fraction of the Musa genome. All repetitive sequences were more abundant in *M. acuminata*. As the genome of *M. acuminata* is larger compared to *M. balbisiana*, the present results demonstrate that the increase in genome size of *M. acuminata* was due to multiplication of some repetitive sequences. The findings of this study improve the knowledge of long-range organization of chromosomes in Musa. The availability of homologous probes for fluorescence in situ hybridization (FISH) will allow more specific mapping of rDNA sequences.

A novel protocol for isolation of high-molecular-weight DNA in Musa has been developed and the work is in progress to construct bacterial artificial chromosome (BAC) library for the B genome of Musa. Availability of the BAC library will permit isolation of clones containing low proportion of repetitive DNA. Such clones will be localized using FISH and will be used to increase the number of already existing chromosome landmarks. Furthermore, BAC library will be screened for clones, which contain the gene for a novel artificial chromosome (BAC) for the B genome of Musa. The availability of homologous probes for fluorescence in situ hybridization (FISH) will allow more specific mapping of rDNA sequences.

The ploidy classification of *Musa* accessions was confirmed by flow cytometry and fluorescent in situ hybridization (FISH).

**Figure 1.** Examples of histograms of relative DNA content, which were obtained during ploidy screening of ITC accessions using flow cytometry. The ploidy of individual plants was estimated based on the ratio of positions of peaks corresponding to G1 nuclei of Musa and CRBC. While the analysis confirmed ploidy classification for *M. acuminata* ssp. banksii (ITC 0885), ‘Lakatan’ (ITC 0573), and TMPx 2637-49 (ITC 1196), the classification was not confirmed for ‘Cavendish 901’ (ITC 0738), which was found to be hexaploid.

![Graph showing ploidy analysis of Musa accessions](image-url)
tain molecular markers and/or genes of interest. This strategy should result in effective integration of genetic and physical maps. Once developed, physically mapped molecular markers will facilitate map-based cloning of genes of interest including those induced by irradiation and chemical mutagenesis.

Ploidy screening of Musa germplasm was supported in part by INIBAP.

Analyses of induced mutants of Philippine bananas (Musa acuminata, cvs ‘Lakatan’ (AAA) and ‘Latundan’ (AAB)) and germplasm collection of Abaca (Musa textilis Nee) using morphological, RAPD, SSR and AFLP markers

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Banana (Musa acuminata) and abaca (M. textilis Nee) are two of the most economically important Musa species cultivated in the Philippines for fruit and fibre, respectively. These crops are both difficult to breed by conventional means and they share a number of common diseases. This report summarizes our efforts during the past five years to generate useful induced mutants of Philippine banana cultivars and to evaluate the usefulness of DNA markers in characterizing genomic alterations in the advanced generations of induced mutants. Furthermore, this report highlights the valuable contribution made by this CRP project in analyzing genetic variation in the related Musa species, M. textilis, by utilizing some of the results of the DNA marker techniques generated for banana. The highlights of our accomplishments are:

- Advanced generations of induced mutants of two Philippine banana cultivars ‘Lakatan’ (AAA) and ‘Latundan’ (AAB) with promising traits were obtained from irradiation using 40Gy gamma ray and 3Gy fast neutron and subsequent in vitro culture manipulation and field evaluation.
- DNA marker techniques such as RAPD, SSR and AFLP were successfully used to characterize genomic differences between the two banana cultivars used. However, only RAPD and AFLP techniques were able to detect genomic alterations between non-irradiated and induced mutants in the two cultivars. Due to better reproducibility and higher multiplex ratio, AFLP technique is preferred over RAPD technique. Hence, this technique was used to detect polymorphism between the original mutated clones and derived suckers. Silver staining procedure for SSR and AFLP analyses were routinely used.
- RAPD, SSR and AFLP techniques developed for banana through this CRP project were found to be highly applicable to abaca. A number of SSR primers developed for banana gave amplification products using abaca DNA. With complimentary funds provided by a grant from the Philippine government, RAPD, SSR and AFLP analyses were successfully conducted to evaluate the genetic variation in the abaca germplasm collection of the Philippines. Comparison between morphological and molecular analyses was also conducted.

Usefulness of embryogenic cell suspensions for the induction and selection of mutants in Musa spp.

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Induced mutation techniques are particularly important for bananas and plantains (Musa species) where there is limited sexual reproduction that could generate genetic variation, the basis for selection. Even though spontaneous mutations have contributed to the genetic diversity of Musa and significantly increased the variation used to breed Musa spp, their occurrence is too low. The use of in vitro cultures for induced mutations in Musa spp. could be a method of choice if several steps of the mutation induction process could be optimized. The following aspects were investigated: the possibility to detect genetic instability in DNA content, the determination of an optimal mutagenic dose, the elimination of chimerism and the application of an early mass screening for the selection of useful mutants. With the increased use of embryogenic cultures in micropropagation of banana, somaclonal variation occurs among regenerated plantlets. This variation may interfere with mutations, which could be obtained through mutation techniques. Although the causes of this chromosome instability are poorly understood, chromosome instability itself is believed to be one of the most common causes of tissue culture-induced variation. Using flow cytometry, variation in chromosome number could be detected among plants regenerated via somatic embryogenesis from tissue culture. The results obtained by flow cytometry were verified by chromosome counting in meristem root-tip cells. After standardization of the method, the results indicated that flow cytometry was sensitive enough to detect aneuploidy in Musa with ≥1 chromosome accuracy. Abnormalities in DNA content could be detected at an early stage, during in vitro culture. For the first time, a banana embryogenic cell suspensions (ECS) with five chromosomess missing was reported.

To irradiate ECS, several preliminary studies were performed. The first radio sensitivity tests of Musa ECS were performed and it has been found that cell suspensions from Musa can tolerate up to 200 Gy. At 100 Gy the growth curve is only affected at 50% compared to the control. When irradiating cell suspensions, large populations can be handled under controlled conditions and if embryos are of single cell origin, they overcome the problem of chimerism. We simulated this by treating ECS with colchicine and determined the ploidy of the regenerated plants by flow cytometric analysis. Colchicine treatment induced polypliody and mixoploidly.
markers (sequence-tagged microsatellite site) was developed for each pathogen, using a microsatellite enrichment strategy. Microsatellites of the (CA)n, (GA)n, and (TA)n type were specifically captured, cloned, positive clones identified by hybridization, sequenced, and microsatellite-flanking primers designed with Primer 3. Together with selective amplification of microsatellite polymorphic loci (SAMPL) and DNA amplification fingerprinting (DAF) markers, the STMS were used to study the genetic variability of both pathogens in populations from two continents, four Latin American countries and five different locations in Colombia. First, all marker types generally detected a high degree of polymorphism in both species. Second, only few markers of any type were sufficient to discriminate two isolates unequivocally. Third, distinct populations can be detected and differentiated from each other. For example, the number of STMS haplotypes turned out to be higher in Nigerian as compared to Mexican M. fijiensis, and the M. musicola populations generally are much more clearly separated from each other than the M. fijiensis populations, pointing to less gene flow. Fourth, usually isolates from one region grouped together. Fifth, about 10% of the primers designed for one pathogen could also be used for the other pathogen. The three marker techniques are used to construct and saturate a first genetic map of M. fijiensis and to tag a fungicide resistance gene.

Genetic and phenotypic variability in Mycosphaerella pathogens of banana

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We are using fungal molecular biology approaches to study the interactions between Mycosphaerella pathogens and their banana hosts. We have developed a DNA-mediated transformation system for M. fijiensis, M. musicola, and M. eumusae in which genetically stable transformants constitutively express green fluorescent protein in vitro and in vivo. Stable transformation is a first step in developing tools for the genetic manipulation of these pathogens, which will lead to the identification of pathogenecity and virulence factors required for plant infection and symptom development. Significant phenotypic variability exists in most Mycosphaerella banana pathogens. When cultured on agar-based media, single colonies are typically light to dark grey or black in colour. Stable spontaneous mutants altered in pigmentation are not uncommon. We have isolated pigment mutants that appear to be deficient in melanin production and have cloned a gene fragment with high similarity to fungal genes involved in melanin biosynthesis. Further characterizations of the pigment mutants are in progress. Additionally, we are interested in whether Mycosphaerella pathogens of banana have the potential to exhibit differential resistance to plant defence responses, such as the generation of reactive oxygen species (ROS), or to themselves produce ROS-generating toxins. We determined that M. musicola produces a singlet oxygen-generating anthraquinone toxin and exhibits significantly greater resistance to a wide range of light-activated, singlet oxygen-generating dyes than does M. fijiensis. These results suggest possible differences in the way the two fungi interact with banana. In M. musicola, the correlation between production of a light-activated toxin, high self-resistance to exogenous photoactivated singlet oxygen-generating toxins, and increased disease development in the presence of light is intriguing and will be examined further. Such a correlation is not evident in the M. fijiensis host-pathogen interaction.

Towards basic genomics of Mycosphaerella fijiensis and M. musicola: DNA markers for genetic diversity, population structure and genetic mapping of the banana pathogens

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Though much research is presently devoted to banana genomics, its two most severe fungal diseases black Sigatoka (causal agent: Mycosphaerella fijiensis) and yellow Sigatoka (causal agent: M. musicola) are definitely under researched. Yet both diseases led, and will lead to major yield losses, especially in banana and plantain plantations, but also on smallholders’ fields. M. musicola first reported in Java in 1902, spread all over the major production areas in Asia, Africa and South America during the first half of the last century. It was regionally displaced by the more aggressive M. fijiensis, first reported on the Fiji islands in 1962. Both pathogens forced to develop control measures, which in essence increased fungicide doses drastically and reduced the time interval between applications, but also relied on the introduction of new, partly resistant host varieties. In response, the pathogen populations became more aggressive and partly resistant to the prevalent fungicides.

In spite of the economic importance of the fungi, systematic research on the pathway of infection, the crosstalk between the pathogen and the host plant, the molecules encountered and the genes involved remained largely elusive. Therefore we started a more basic study on both pathogens with the aim to understand the molecular basis of the interaction(s).

A set of elite, highly polymorphic microsatellite markers (sequence-tagged microsatellite site
A study was done with the objective of developing a low-cost DAS-ELISA detection kit. Anti-serum for BBrMV (of Queensland Department of Primary Industries - QDPI, Australia) was tested as the coating antibody to replace the relevant component of the Agdia commercial kit. Results showed a relatively high efficiency with the QDPI antibody. Work is also in progress to make the alkaline phosphatase enzyme conjugated antibody to substitute the one of the test kit. Once the local antisera is produced, it is expected that an effective and low cost local diagnostic kit will be developed for the routine indexing of banana plants for BBrMV. Purification of the virus extract (Thomas et al. 1997) is a limiting factor for obtaining the antigen for the antibody production process.

References

Advances and perspectives for the genetic improvement of banana (Musa spp.) via biotechnological and nuclear techniques at INIVIT

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Bananas and plantains constitute an important carbohydrate source in the Cuban diet. This is mainly due to their food habits and banana production all the year round. There is a great need to develop new banana cultivars due to poor yield and susceptibility to diseases (mainly black Sigatoka caused by the fungus Mycosphaerella fijiensis). The application of both biotechnological and nuclear techniques has enabled to develop an efficient plant regeneration system, and to induce genetic variability for mutant selection. However, the success in obtaining desirable mutants is restricted to a few allele combinations and thus, more efficient alternatives are needed for an early mutant selection. Earlier, clones obtained by gamma irradiation of meristematic apices, LD50 dose obtained in Seibersdorf (Austria) and the Centro de Estudios Aplicados al Desarrollo de la Energía Nuclear (CEADEN) in Cuba, were tested in field conditions. Somatic embryogenic cell suspension cultures were established and the somatic embryo formation is being further modified to enhance further plant regeneration rate, especially of AAB genotypes in collaboration with KU Leuven. Preliminary evaluations were carried out to study the action of crude extract of M. fijiensis on cell suspension cultures of ‘Navolean’ in a solid ZZ medium. Filter paper discs were used to test different concentrations of crude extract on cell culture growth for selecting toxin tolerant cell cultures. Effect of fungal crude extract on oxygen uptake studies were made in ‘CEMSA’ clone, as well as in vitroplants of different cultivars used in the IMTP for black Sigatoka studies. The vitroplant leaves were treated with different concentrations of crude extract solution of ethyl acetate by placing them on different sizes of disks, e.g. starting from 0.28 cm² size disks. Oxygen uptake was measured by Warburg’s manometers. Based on our results, a short stature mutant (‘Parecido al Rey’ 6.44) was obtained. Few more mutants were selected, which were tolerant to black Sigatoka with increased yield, in some cases (‘Parecido al Rey’ 6.32 and ‘Gran Enano’ 6.44 mutants and III-2). However, these characters were unstable, probably affected by the environment, or in vitro culture conditions. Therefore, we looked for other alternative approaches for improving induced mutation rates, e.g. somatic cell suspension cultures and later mass of somatic embryogenic cells were established. From each batch of somatic embryogenic cell suspension mass cultures, 3250 to 6625 somatic embryos were obtained with 20.7% germination and 95% plant survival in ex vitro conditions. Their genetic stability is presently being tested in field conditions. The action of M. fijiensis extract on cell suspension culture resulted in large number of oxidized cells at higher extract concentrations. Damaged cells were characterized by a compact cytoplasmic content with a dark colour in its centre and leaving an empty space between it and the cellular wall. Forty-five days after incubation, more than 60% cells were found in the previous described conditions due to toxin diffusion from discs. Oxygen uptake valves had decreased normally in non treated control of all IMTP cultivars, and the maximum decrease observed in treated plants in comparison with non-treated plants was: ‘Yangambi km5’ (38.7%); ‘Calcutta 4’ (27.0%); ‘Pisang berlin’ (13.4%); ‘Pisang illin’ (20.9%) while oxygen uptake was increased by 70% in ‘CEMSA’ clone. The cultivars with higher resistance to
Agrobacterium-mediated cotransformation of banana (Musa spp.)

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The introduction of foreign genes into the plant genome is a basic technique to study gene expression and physiological processes in plants and for breeding programmes. Improving the agronomic value of major crops is likely to involve the introduction of multiple genes, many of which will not provide directly screenable phenotypes among the initial products of transformation. Major restrictions of current transformation techniques are that only a few genes can be transferred at the same time and that selectable marker genes have to be used, which frequently results in transgenic plants containing undesirable antibiotic resistance genes.

The objective of the present study is to determine the efficiency of cotransformation with visually scarable marker genes using Agrobacterium tumefaciens and banana (Musa spp.). Cell suspension culture of four cultivars were infected with two different A. tumefaciens strains each carrying a distinct disarmed T-DNA containing one of three reporter genes [Luciferase (LUC), B-Glucuronidase (GUS) or Green Fluorescent Protein (GFP)] as well as the neo selectable marker gene. Multicellular structures expressing multiple genes were recovered, and cotransformation frequencies were measured. The cotransformation frequency was less than the sum of the frequency of each single transformation. Negative correlation was found between the transient expression of two visual marker genes introduced together for cotransformation. Significant differences in (co-)transformation frequency were detected between the banana cultivars tested.

We anticipate that the simultaneous use of multiple reporter genes will provide a convenient method for the accurate determination of cotransformation and will contribute to a strategy for multigene transformation.

Recent developments in early in vitro screening for resistance against migratory endoparasitic nematodes in Musa

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Among nematodes parasitizing bananas throughout the world, Radopholus similis and Pratylenchus coffeae are important migratory nematodes, causing severe yield losses in commercial and local consumption cultivars. Chemical control is currently the most used method to manage the nematodes although nematicides are dangerous, toxic, and expensive. Therefore, nematode control through genetic improvement of banana is widely encouraged. Many Musa cultivars have been screened to find resistance against these root pathogens. Screening research is time consuming because it must be carried out both under field and greenhouse conditions. In vitro screening could facilitate and hasten incorporation of genetic nematode control into bananas. However, an in vitro screening method requires aseptic nematode cultures. In this paper, the development of aseptic cultures of R. similis and P. coffeae and an in vitro screening method are discussed. Alfalfa callus on modified White’s medium has proved to be a good aseptic culture system for both R. similis and P. coffeae. Although the reproduction is significantly lower compared to carrot disc cultures, this system has many advantages. The nematodes are not only cultured under complete aseptic conditions but this system is also less labour intensive and offers a more continuous inoculum production. In addition, culturing on alfalfa callus did not alter the pathogenicity of R. similis and P. coffeae. Both R. similis and P. coffeae could infect and reproduce on the roots of in vitro grown ‘Grande naine’ plants. For both nematodes necrotic lesions were observed in the roots within 2-3 weeks after inoculation. In a last experiment, the reproduction of R. similis was tested in vitro on six different Musa cultivars with a known host response to R. similis. Except for ‘Yangambi km5’, their host response under in vitro conditions corresponded to their host response under greenhouse or field conditions. The susceptible status of ‘Grande naine’, ‘Gros Michel’ and ‘Cachaco’ was confirmed as well as the resistant status of ‘Pisang jari buaya’ and ‘SH-3142’.

Evaluation for nematode control of transgenic plants expressing different types of lectins

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In the vast majority of African countries where starchy bananas and plantains are grown, Musa spp. are important staple food crops, often exclusively grown by small scale farmers. This especially applies to the highland cooking and brewing bananas of East and Central Africa. Over the last two decades, however, banana yields in the East African region have been steadily declining partly due to migratory or sedentary endoparasites: the burrowing nematode (Radopholus similis), the root-lesion nematodes (Pratylenchus coffeae) and knot nematodes (Meloidogyne spp.). With the advent of transgenic methodologies, an attractive method to control these nematodes is the transfer into target Musa spp. of lectin encoding genes. Prior to application in banana, the nematocidal effect of a number of lectins or lectin-related proteins is currently tested for their effectiveness against banana nematodes in transgenic Arabidopsis thaliana and/or tobacco. The different in vitro test systems will be discussed, together with the initial results.

Lectin binding to the plant parasitic nematode Radopholus similis and its effect on host finding

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The effect of lectins on the plant parasitic nematode Radopholus similis was studied in a series of experiments. FITC- or colloidal gold-labeled lectins of Canavalia ensiformis (ConA), wheat (WGA) and Helix pomatia (UPA) were found to bind the nematode in the head region, at the excretion pore, the pores of the reproduction system and those of the phasmids. The viability and the chemotactic response towards plant roots, after treatment of nematodes with lectins, were examined in vitro by analyzing movement tracks left on agar plates. The assay included six plant lectins of different classes and the banana thauatin-like protein. A 1% concentration of Phaseolus vulgaris agglutinin (PHA) had a toxic effect on R. similis females: 68% showed no or very little movement after inoculation compared to an average of 30% for other lectins and 5% for the control treatment. A 0.05% concentration of PHA still reduced the viability of R. similis females by 75%. ConA and WGA did not alter the chemotactic response towards plant roots, despite the demonstrated binding of both lectins to R. similis. In contrast, Galanthus nivalis agglutinin (GNA) reduced orientated movement of R. similis females by 75%.

In the last experiment, the reproduction of R. similis was tested in vitro on six different Musa cultivars with a known host response to R. similis. Except for ‘Yangambi km5’, their host response under in vitro conditions corresponded to their host response under greenhouse or field conditions. The susceptible status of ‘Grande naine’, ‘Gros Michel’ and ‘Cachaco’ was confirmed as well as the resistant status of ‘Pisang jari buaya’ and ‘SH-3142’.

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Early detection of dwarf off-types of banana using AFLP, TE-AFLP and MSAP analysis

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AFLP and several variant techniques were performed on banana in order to characterize different dwarf phenotypes. The dwarf AAB plantain ‘Curare enano’ and its normal-sized in vitro generated off-type were analyzed by AFLP, TE-AFLP, MSAP, cDNA-AFLP, and cDNA-TE-AFLP. AFLP and TE-AFLP were also performed on four pairs of naturally occurring dwarf normal banana cultivars. Differential AFLP patterns were obtained and up to 25% polymorphism was observed depending on the primer combination and the cultivar. TE-AFLP analysis generated shorter and a lower number of fragments resulting in only relatively few polymorphisms between the dwarf and normal-sized cultivars. The somaclonal variants obtained in vitro from the dwarf ‘Curare enano’ might have been caused by methylation induced by in vitro conditions. MSAP analysis, based on the methylation (in)sensitivity of a pair of isoschizomeric restriction enzymes, appeared to be a valuable tool in revealing differential cytosine methylation. Cloning and sequencing differential fragments did not reveal significantly homologous matches in public databases. cDNA-AFLP analysis between the dwarf and normal ‘Curare enano’, revealed a normal-specific fragment length polymorphism (AFLP), to characterize the genomic alterations in somatic meristems and to discriminate between closely related genotypes. More primer combinations and/or alteration of restriction enzymes will increase the chance of finding more dwarf-related sequences.

Establishment of embryogenic cell suspension cultures from Indian banana cultivars

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Banana (Musa spp.) is an important and major fruit crop in India, which is the largest producer of bananas in the world. However, diseases and pests such as black Sigatoka and Panama disease, bunchy top virus and nematodes remain as major threats to production. Genetic modification using embryogenic cell suspensions (ECS) appears to be a suitable approach for integrated genetic improvement. Progress has been made in the development of protocols for the establishment of ECS, and immature male flowers as well as proliferating in vitro cultures have mainly been used. Especially in vitro proliferating meristems (‘scaps’) are an ideal starting material, because they can be generated all round the year from most cultivars. The method includes a preculture of the proliferating meristems on high cytokinin medium which provides embryogenic competence. Several important Indian cultivars [‘Robusta’ (AAA), ‘Basrai’ (AAA), ‘Shrimanthi’ (AAA), ‘Karpaora velli’ (ABB)] have been employed in this study for the induction of good quality scaps and embryogenic callus. Of these, ‘Robusta’ have shown the formation of embryogenic callus, which subsequently has been used for the establishment of ECS. In addition, the effect of alternative cytokinins such as meta-topoline (MT), thidiazuron (TDZ) and N-chloro-4-pyridyl-N-phenyleurea (CPPU) has been studied on isolated meristems of ‘Cachacco’ (ABB), ‘Williams’ (AAA) as well as on scaps of ‘Robusta’. On isolated meristems (‘Williams’, ‘Cachacco’), CPPU has only resulted in the development of single shoots and roots, whereas MT resulted in the formation of watery callus and proliferation. TDZ mainly induced swelling of the explants. TDZ proved to be better cytokinin over MT in the induction and maintenance of good quality scaps. These are currently under evaluation for embryogenic induction. The established ECS will be characterized and used in cryopreservation and genetic transformation experiments.

Table 2. Summary of polymorphism detected in AFLP markers used in the analysis of irradiated parent clones, first cycle suckers and non-irradiated clones of cultivars Lakatan and Latundan

<table>
<thead>
<tr>
<th>Selective primer pairs</th>
<th>No. of bands</th>
<th>Number of polymorphic bands</th>
<th>% Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-AGC/M-CTC</td>
<td>35</td>
<td>11</td>
<td>31.4</td>
</tr>
<tr>
<td>E-AGC/M-CTG</td>
<td>32</td>
<td>20</td>
<td>62.5</td>
</tr>
<tr>
<td>E-AGC/M-CTT</td>
<td>18</td>
<td>14</td>
<td>77.8</td>
</tr>
<tr>
<td>E-AGC/M-CAA</td>
<td>40</td>
<td>20</td>
<td>50.0</td>
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<tr>
<td>E-AGC/M-CAC</td>
<td>34</td>
<td>14</td>
<td>41.2</td>
</tr>
<tr>
<td>E-AGC/M-CAG</td>
<td>29</td>
<td>14</td>
<td>48.3</td>
</tr>
<tr>
<td>E-AGC/M-CTA</td>
<td>31</td>
<td>14</td>
<td>45.2</td>
</tr>
<tr>
<td>E-AGC/M-CTC</td>
<td>21</td>
<td>7</td>
<td>33.3</td>
</tr>
<tr>
<td>E-AGC/M-CTG</td>
<td>29</td>
<td>19</td>
<td>65.5</td>
</tr>
<tr>
<td>Mean</td>
<td>34</td>
<td>15.1</td>
<td>51.1</td>
</tr>
</tbody>
</table>
are otherwise morphologically indistinguishable. The results indicate that AFLP is very ideal and useful for fingerprinting purposes compared with other marker systems because of its high multiplex ratio i.e. more bands (=loci) per gel can be resolved. While more primer combinations need to be tested, these results suggest the potential usefulness of this technique in detecting genome variation between cultivars and in detecting genome alterations in induced mutants of banana including those showing very similar phenotypes. The detected variation between the irradiated parent clones and suckers suggests that the number of vegetative propagation cycles for the shoot-tip technique used (Novak et al. 1989) may not be sufficient to completely eliminate chimeras in the mutated populations. The results obtained could provide a sound basis for more successful application of mutation and molecular marker techniques for improvement of banana in the Philippines.

**Reference**


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**Rat feeding tests of transgenic banana expressing an antifungal peptide from onion**


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Transgenic plants of the dessert banana ‘Williams’ containing a gene encoding the Ace-AMP1 antifungal peptide of onion (Allium cepa) were produced by particle bombardment for enhanced tolerance against attack by the fungal pathogen Mycosphaerella fijiensis. ELISA assays on extracts from lyophilized banana pulp showed that the concentration of the Ace-AMP1 peptide reached up to 0.0316% of the total amount of soluble proteins or six times above the background signal measured in non-transformed banana pulp. We tested whether this expression level had an effect on rats fed on a diet containing transgenic banana pulp.

While energetic content was comparable in transgenic and control pulp, dry matter and protein content were lower and higher in transgenic pulp than in control pulp respectively. Twenty per cent of lyophilized meal from control or transgenic bananas were incorporated in regular rodent food and supplied to male and female Wistar rats. Feeding of the transgenic meal during six weeks did not cause any difference in food intake, growth rate and weight of internal organs in comparison to feeding on control diet. Also, a complex blood analysis did not show any effect in rats consuming the transgenic banana meal.

**Cryopreservation for the elimination of cucumber mosaic and banana streak diseases in banana (Musa spp.)**

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Bananas and plantains (Musa spp.) are threatened by various pests and diseases, including important viral diseases which constrain banana production and cross-border germplasm movement. This delays dramatical for high yielding and newly bred varieties to small farmers. INIBAP therefore established a system for the safe international movement of Musa germplasm. This involves that all germplasm

held at the international Musa germplasm collection of INIBAP (based at KU Leuven, Belgium) is tested by different virus indexing centres (South Africa, Australia and France). Currently about 25% of the collection comprising a significant number of potentially important and improved varieties is infected with viruses. The most prevalent viruses in this infected germplasm are BSV (banana streak virus) but also with CMV (cucumber mosaic virus), BBTV (banana bunchy top virus), BanMMV (banana mild mosaic virus) and BRMV (banana racket mosaic virus).

A programme of virus elimination is therefore carried out by the Plant Pathology Unit (FUSAGx, Belgium) in collaboration with the Laboratory of Tropical Crop Improvement (KU Leuven, Belgium). Different in vitro techniques, such as thermotherapy, chemotherapy, meristem culture and cryotherapy are tested. Banana plants of the cultivar ‘Williams BSJ’ (AAA) were either naturally infected with BSV or mechanically infected with CMV or BBTV. Proliferating in vitro cultures were produced from this material. Excised meristematic clumps were cryopreserved through vitrification using the PVS-2 solution. The health status of regenerates material was first checked on in vitro plants through ELISA. Then, the putative virus-free material was tested again after greenhouse acclimatization. The virus eradication rates after cryopreservation were 30% and 90% respectively. In comparison, the frequency of virus-free plants regenerated directly from highly proliferating meristems, which reflects spontaneous eradication, reached 0% and 52% for CMV and BSV respectively. The conventional meristem culture resulted in 0% CMV-free plants and 76% BSV-free plants.

In conclusion, cryopreservation seems to be a very promising technique for virus eradication from Musa germplasm enabling to faster distribute germplasm of interest.

**An ultrasensitive luminescent detection system for banana biotechnology: from promoter tagging to Southern hybridization**

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Digital imaging using a cooled CCD camera is becoming an increasingly versatile tool in biotechnology research. An ultrasensitive camera system consisting of a liquid nitrogen cooled slow-scan CCD camera connected to powerful image analysis software is able to detect very low levels of light emission from several signal sources and is used for the recording of results for different applications in banana biotechnology.
The most sensitive reporter system, bioluminescent luciferase (LUC) is used in T-DNA mediated promoter tagging. Since integration of the promoterless luc gene is random, the level of LUC expression is suboptimal in most transformants to be screened requiring a highly sensitive detection. Preliminary results on in vivo screening of LUC expression in hundreds of putative promoter tagged cell cultures will be presented.

Chemiluminescence has been for several years the method of choice in our laboratory for non-radioactive hybridization analysis. Although exposure to and development of an X-ray film is a sensitive technique, it is time consuming and costly because multiple exposures are needed for the evaluation of results. In addition to increased flexibility and higher sensitivity of detection, signals can be captured faster with the CCD camera than with film. Good results can be obtained with a single exposure by adjusting the gray scale of the captured image as will be demonstrated.

In addition to luminescence the camera can also detect fluorescent signals, which is demonstrated by the ability to monitor green fluorescent protein expression in transgenic banana cultures. Quantification of light intensity by software analysis will be demonstrated.

**Agrobacterium- and particle bombardment transformation of a wide range of banana cultivars**

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Genetic transformation of banana (Musa spp.) by particle bombardment and Agrobacterium is established only in a few laboratories worldwide. In general, transformation frequencies are reported to be cultivar dependent. Thus, there is a need to optimize established transformation protocols for any particular type of banana. In this study, the two transformation methods were compared and the effect of physical parameters on transformation frequency was investigated in four banana cultivars: ‘Grande naine’ (AAA), ‘Obino I’ewai’ (AAB), ‘Orishelle’ (AAB), and ‘Three hand planty’ (AAB). DNA transfer frequency was determined by the ability to monitor green fluorescent protein expression in transgenic banana cultures. Quantification of light intensity by software analysis will be demonstrated.

**Cryopreservation of embryogenic cell suspensions of banana to support banana improvement**

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The initiation of embryogenic cell suspension cultures of banana is still difficult and time-consuming, irrespective of the starting material used (immature male flowers, immature zygotic embryos or proliferating in vitro meristems). The embryogenic response is very low and slow. Indeed for most cultivars less than 1% of initial explants give rise to an embryogenic callus suitable for cell suspension initiation and 9-26 months are needed before such embryogenic cell suspension is established. Moreover, once established, these cell suspensions are subject to somaclonal variation and microbial contamination and a prolonged culture period may result in a lower and eventually a total loss of morphogenic capacity.

Up to now, transformation protocols of banana rely on embryogenic cell suspensions. Particle bombardment as well as Agrobacterium-based protocols resulted in transgenic banana plants. Somatic hybridization and protoplast electroproporation are also both depending on the isolation of regenerable protoplasts from embryogenic cell suspensions. Finally, embryogenic cell suspensions can be used for mass propagation as an alternative to shoot-tip cultures.

The safe preservation of this valuable suspensions through cryopreservation is thus of outmost importance. A cryopreservation technique was developed which involves cryoprotection with 7.5% DMSO (dimethyl sulfoxide) and 180 g/L sucrose, followed by slow freezing at 1°C/min to -40°C and plunging into liquid nitrogen. Currently, 651 cryotubes containing embryogenic cell suspensions belonging to 48 independent cell lines and 14 different cultivars are safely stored in liquid nitrogen for the long term. Recently, banana cell suspensions were recovered after five years storage in liquid nitrogen. The ability to produce somatic embryos remained intact. Also their competence towards Agrobacterium-mediated transformation was screened and compared to a non-cryopreserved cell suspension of the same cell line. The transient expression of the introduced marker gene as well as the regeneration efficiency of transgenic plantlets was comparable.

**Oxidative events induced by Mycosphaerella fijiensis metabolites in banana (Musa spp.) black leaf streak disease and analysis of early selection feasibility for resistance to this disease**

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The mechanisms of action of M. fijiensis toxins in black leaf streak (BLS) disease were studied. The ethyl acetate crude extract (EaCE) from the pathogen culture filtrates and juglone (5-hydroxy-1,4-naphthoquinone), which is a purified metabolite from EaCE, were injected in the leaves of two banana cultivars. The cultivars ‘Grande naine’ and ‘Fougamou’ served as a susceptible reference and partially resistant reference respectively. These bioassays induced necrosis and showed decrease of the vitality index determined according to chlorophyll fluorescence data (Lichtenthaler et al. 1986). The ‘Grande naine’ cultivar was more sensitive than Fougamou whatever the bioassay (induction of necrosis or chlorophyll fluorescence) taken into account. The light-dependence of the toxicity revealed by these tests, the early effect on chlorophyll fluorescence (Harelmlama et al. 1997) and the swelling of ‘Grande naine’ chloroplasts after injection with EaCE, are indicative that chloroplasts could be a potential target site for M. fijiensis toxins.

A mechanical protocol (Leegood and Malkin 1986) to isolate physiologically intact chloroplasts from banana leaves was developed. A new bioassay based on the addition of juglone to banana chloroplast suspensions was used to analyse the impact of M. fijiensis metabolites. By performing the Hill reaction (Allen and Holmes 1986) with the so treated suspension to measure the ability of chloroplasts to transfer electrons, a direct inhibiting effect of juglone on this physiological activity was clearly demonstrated. Moreover, this effect was again higher with ‘Grande naine’ chloroplasts than with those of ‘Fougamou’.

Since chloroplasts constitute one of the sites of active oxygen species production in plants (Sutherland 1991, Foyer et al. 1997), their direct interactions with juglone in bananas led to a new hypothesis. Hence, oxidative events were suspected to be at the origin of the physiological damages in the isolated chloroplasts. In fact, involvement of fungal naphthoquinone metabolites in oxidative process in not uncom-
mon (Medentsev and Akimoto 1998). Their auto-oxidative property responsible of the oxidation of NADH and NADPH leads to the removal of these molecules from the oxidative phosphorylation system as potential sources of reduction equivalents for the respiratory chain.

In the case of BLS disease, assessment of this hypothesis was performed by considering possible interactions between juglone and banana antioxidant systems. We observed that juglone causes an in vitro oxidation of ascorbic acid, the most abundant antioxidant in plants (Smirnoff 2000). The occurrence of oxidative phenomena induced by this metabolite in bananas was also assessed by analysing the superoxide dismutases (SOD) patterns at several intervals of time following juglone injection into leaves of the two reference cultivars. In fact, superoxide dismutases are assumed to play a central role in the defence against oxidative stress (Beyer et al. 1991, Scandalias 1993). Our preliminary observations showed that there was a repressive effect on one SOD isoform in ‘Grande naine’ while a stimulating effect on another SOD isoform in ‘Fougamou’ seemed to occur. On the base of the results obtained with the two antioxidant systems analysed previously, juglone could be supposed to deprive bananas partly of their antioxidant capacity. Further investigations have to be done in this area in order to determine exactly all the mechanisms affected by the pathogen metabolites during BLS development.

Finally, a first in vitro selection assay for BLS resistance was also performed. Therefore, different juglone concentrations were mixed with embryogenic cell suspensions of two lines of THP (‘Three hand planty’ cultivar) as well as with suspensions containing somatic embryos of the same cell lines. After an overnight incubation, the material was transferred onto juglone-free fresh media. In general, both plant materials necrosed and did not show any weight increase during the period of incubation following the treatment. However, from somatic embryos of one treated line, some plants were obtained from tissues that did not become necrotic with 50 ppm of juglone. These regenerated plants are going to be evaluated for their eventual BLS resistance with the abovementioned bioassays as well as by artificial inoculation of the pathogen. These plant regenerates will constitute a precious material to further analyse the role played by *M. fijiensis* toxins in development of BLS disease.

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