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IN THIS ISSUE

In situ mass propagation of FHIA-20 using benzylaminopurine

Socioeconomic aspects of plantain cultivation in Colombia

Production of plantain leaves for the agrifood industry

The evolution of photosynthesis, transpiration and chlorophyll during the development of leaves of plantain

Estimation of root development from shoot traits in Musa spp.

Evaluation of cultural, chemical and biological control of vascular rot and wilt in plantain

Evaluation of FHIA hybrids in comparison with local Musa clones in Peru

Evaluation of Musa germplasm against banana weevil borers

Distribution of Fusarium wilt of banana in Kenya and its impact on smallholder farmers

VCG of the populations of Fusarium (Foc) in Vietnam

Black Sigatoka disease in Mexico

Effect of number of subcultures on in vitro multiplication of banana clones

Musa News

The banana world loses two friends and colleagues

INIBAP News

Thesis

Books etc.

Announcements

PROMUSA News

ICTA

INFOUSA is published with the support of the Technical Center for Agricultural and Rural Cooperatives (ICTA)
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.

CONTENTS

*In situ* mass propagation of the FHIA-20 banana hybrid using benylaminopurine ........................................... 3

Socioeconomic aspects of plantain cultivation in Colombia .............................................................. 4

The production of fire-softened plantain leaves for the agrifood industry ..................................... 9

The evolution of photosynthesis, transpiration and chlorophyll during the development of leaves of plantain (*Musa* AAB Simmonds) .................................................. 12

Estimation of root development from shoot traits in plantain and banana (*Musa* spp.) .................. 15

Evaluation of cultural, chemical and biological control of vascular rot and wilt in plantain (*Musa* AAB Simmonds) ................................................................. 17

Evaluation of FHIA hybrids in comparison with local *Musa* clones in a black Sigatoka-free area of eastern Peru ................................................................. 21

Evaluation of *Musa* germplasm against banana weevil borers ............................................. 26

Distribution of Fusarium wilt of banana in Kenya and its impact on smallholder farmers ............ 28

Vegetative compatibility groups of the populations of *Fusarium oxysporum* f.sp. *caeruleum* in Vietnam .................................................................................. 32

Black Sigatoka disease (*Mycosphaerella fijiensis*) in Mexico ....................................................... 33

Effect of number of subcultures on *in vitro* multiplication of four banana clones .................. 38

*Musa* News ................................................................................................................................. 40

The banana world loses two friends and colleagues ........................................................................ 40

INIBAP News ............................................................................................................................ 42

Thesis ........................................................................................................................................ 47

Books etc. ................................................................................................................................. 47

Announcements ....................................................................................................................... 50

PROMUSA News .................................................................................................................. I to XVI
In situ mass propagation of the FHIA-20 banana hybrid using benzylaminopurine

D. Manzur Macias

Bananas and plantains are giant perennial grasses resulting from intra- and interspecific hybridization of two diploid forest species: Musa acuminata (banana) and M. balbisiana (plantain). They flourish in tropical regions and are the most important carbohydrate source in local economies (Stover and Simmonds 1987). The most alarming phenomenon for banana and plantain cultivation has been the appearance and spread of diseases such as black Sigatoka (Mycosphaerella fijiensis Morelet) and those caused by the streak mosaic virus (BSV) and cucumber mosaic virus (CMV). These problems have been tackled by breeding programmes which have succeeded in developing plantain varieties resistant to black Sigatoka (Vuylstekke 1998), with a high yield and high palatability. The FHIA-20 hybrid bred by Dr Phil Rowe at Fundación Hondureña de Investigación Agrícola (FHIA) is an example.

Improved plantains are polyploid and parthenocarpic and are thus propagated vegetatively from daughter suckers obtained from mother-plants just before harvesting. The dormant buds located on the rhizome of the mother-plant are stimulated after bunch harvesting, by cutting the rhizome into pieces or by removing the base of the leaf sheaths and dissecting the developed buds (Aboiron 1997). In vitro mass propagation, or micropropagation, is performed routinely based on the proliferation of apical meristems cultivated on Murashige & Skoog medium enriched with cytokinins and vitamins (Krikorian and Cronauer 1984). One of the commonest limiting factors for the extension of a plantation of plantain banana is the difficulty in obtaining planting material, owing to the very nature of the plant, its limited sucker production and slow development (Tézenas du Montcel 1985).

The present study is aimed at evaluating an in situ multiplication technique for FHIA-20 plantain.

![Figure 1. Differentiation of first-generation suckers (G1S).](image)

Materials and methods

FHIA-20 hybrid tissue culture plants from FHIA were multiplied by micropropagation in the tissue culture laboratory of the Plant Technology Department, using the protocols developed by various authors (Ma and Shii 1972, Hwang et al. 1984). The plantlets obtained, after becoming acclimatized to field conditions under intermittent misting, were transplanted to their final location at Montelindo Farm (owned by Caldas University), located 5°5′N and 75°40′W at an elevation of 1050 m with average temperature of 23°C and Typic Distrandept soils, on a 25-plant plot surrounded by Dominico hartón plantain with a 2 x 3 m plant lay-out in furrows. The plants were fertilized one month after planting according to the results of soil analyses and nutritional requirements of FHIA-20 plant material.

Ten months after planting, each plant had developed 8 to 10 suckers, reaching a height of 15-20 cm and a diameter of 15-20 cm at the collar of the rhizome. These are the first-generation suckers (G1S) (Figure 1).

Using a knife disinfected with formal 2% before each operation, the pseudostem of each sucker was cut transversely 2 cm above the collar of the rhizome and the apical meristem was removed at a depth of 4 cm, leaving a 2 cm-diameter cavity in the rhizome (Figure 2A); the pseudostem fragment was then cut with crosswise incisions, boring down to the rhizome collar (Figure 2B). Once these cuts had been made on each sucker, 4 ml of benzylaminopurine (BAP) at 40 mg/l distilled water were deposited in the cavity left by the removal of the apical meristem (Figure 2C). The rhizomes were then covered with a mixture of equal parts of sandy loam and poultry dung compost to 5 cm above ground level. The so-called second-generation suckers (G2S) appeared after 3 months on each treated sucker (Figure 2D).

When the suckers originating from G2S had differentiated and reached a height of 20 to 30 cm, they were dissected again using the procedure described above, the same quantity of BAP was placed in the cavities and the operation was completed in the same way (Figure 3A) until third-generation suckers (G3S) were obtained (Figure 3B).

Sixty days later, the G3Ss were treated in the same way as the preceding generations to obtain fourth-generation suckers (G4S) that were left to grow (Figure 4A) for ulcerating planting and rooting in sterile soil under intermittent misting (Figure 4B).

Results

This in situ mass propagation technique (extraction of apical meristem, cross-shaped cutting and addition of BAP) gives an average of four suckers at the G1S and G2S stages; the same
Socioeconomic aspects of plantain cultivation in Colombia

J. L. Rodríguez Martínez and A. Rodríguez Saavedra

Plantain cultivation has become a feature of great socioeconomic importance in Colombia from the point of view of food security and job creation. In addition, plantain belongs to the traditional sector of the rural economy, where it is used mainly to shade coffee and is an essential component of the diet. More than half of the cultivated area in Colombia belongs to small farmers (Rodríguez Saavedra et al. 1999).

In the agricultural sector, plantain is in fifth position after coffee, sugar cane, potatoes and flowers. It accounts for 6.8% of the country’s total agricultural production (OCI 2000).

Plantain is grown in different agroecological zones from sea level to 2000 m and at temperatures ranging from 17 to 35°C. Some 358,000 ha of plantain is cultivated producing an annual 2.5 million tonnes of fruits, of which 95% is sold on the domestic market and the rest is exported. The main production centres are in the coffee production zones in the Andean region where 231,000 ha is under plantain (64% of the total cultivated area), accounting for 67% of national production. Other important natural regions for plantain are the...
Orinoco, the Pacific, the Caribbean and Amazonia.

Of the area cultivated, 87% is devoted to traditional cultivation with coffee, cocoa, yuca and fruit trees and the remaining 13% is used for mechanized monoculture (Rodríguez Saavedra et al. 1999).

The central coffee zone supplies most of the main markets in the country. The ‘Domínico hartón’ clone is the variety most commonly planted in this region. ‘Hartón’ is the main clone in other production regions such as the Caribbean, the Orinoco, the Pacific and Amazonia because it is more suitable and productive in zones below 1000 m (Rodríguez Saavedra et al. 1999).

According to Corporación Colombia Internacional, the consumption of fresh plantain was estimated at 62 kg per person per year in 1999, one of the highest figures in the world.

The present state of plantain cultivation

In the world

For agroclimatic reasons, plantain growing is concentrated in Africa, Latin America and the Caribbean.

Table 1 shows that in 1999 the world plantain area totalled 4.8 million hectares producing 30.6 million tonnes of fruits. The regions with the largest production are in Africa and Latin America with respectively 74.2% and 22.5% of world production in comparison with 3.3% in Asia.

The four main producer countries in Africa are, in descending order, Uganda, Rwanda, Ghana and Nigeria; the main producers in Latin America and the Caribbean are Colombia and Peru and, finally, Sri Lanka is the main producer in Asia.

Colombia accounts for 39.1% of production in Latin America and the Caribbean and 8.8% of world production. These figures have been fairly stable for the past eight years. Peru follows with 4.4% of world production and 19.5% of that of Latin America and the Caribbean.

World consumption

The greater part of world plantain production is aimed mainly at covering the domestic requirements of the producer countries. Only 1% is sold on international markets to meet the demand from consumers of Latin American origin and, to a lesser extent, of African origin (CCI 2000).

It is estimated that 10% of the plantains imported by the United States are used for derived products whose consumption increased by 15% from 1991 to 1995. This type of product is still aimed at communities of Latin American or African origin, but it is sought to target consumers of Anglo-Saxon origin since they form the majority of the population of North America, making it one of the most sought-after markets for plantain exporters. The following companies account for 90% of the market: Marigua, Micranda, Chips, Goya Foods and Chiffles Chips (CCI 2000).

In the European Union, the Netherlands, Belgium and Spain are the main importing countries and in turn export the produce to other EU members. The European market for green plantain is limited and fairly stable since demand is only from Latin American, Caribbean and African communities. The main supplier countries are Colombia and Costa Rica, although some African countries also make a marginal contribution to market supplies (CCI 1998).

Table 1. World plantain production in 1999 (FAO 1999).

<table>
<thead>
<tr>
<th>Region</th>
<th>Area (10^4 ha)</th>
<th>Yield (t/ha)</th>
<th>Production (10^4 t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin America and the Caribbean</td>
<td>830.7</td>
<td>8.3</td>
<td>6,898.0</td>
</tr>
<tr>
<td>Africa</td>
<td>3,966.5</td>
<td>5.7</td>
<td>22,706.7</td>
</tr>
<tr>
<td>Asia</td>
<td>89.0</td>
<td>11.3</td>
<td>1,013.3</td>
</tr>
<tr>
<td>Total</td>
<td>4,866.2</td>
<td>6.2</td>
<td>30,618.0</td>
</tr>
</tbody>
</table>

Importing countries

The United States, Europe and Japan are the main importers of plantain, purchasing 80% of exports. The United States imports plantain only from Latin America and the Caribbean—from Colombia, Ecuador, Venezuela, Costa Rica and the Dominican Republic among others. Japan imports from the Philippines, China and South Africa whereas the European Union imports plantain from its former colonies and also from Latin America and the Caribbean. Europe also produces what is commonly referred to as ‘community plantain’ from Spain, Portugal, Greece and French overseas departments such as Martinique and Guadeloupe (Rodríguez Saavedra et al. 1999).

Exporting countries

Colombia is considered as being the main plantain exporter to the United States and European Union markets, with slow growth in terms of the volumes exported. In 1995, plantain exports totalled 105,000 tonnes worth US$ FOB 36 million. The figures in 1998 were 121,000 tonnes worth US$ FOB 42.1 million, a growth rate of 4.9%. Export shipments from Colombia to the United States increased from 80,000 tonnes worth US$ CIF 28 million in 1992 to 109,000 tonnes worth US$ CIF 40.4 million in 1999, representing 4.6% growth in export volumes.

Ecuador is the second exporting country after Colombia. Exports to the United States have decreased considerably in the past eight years, with 7.3% average variation. The smallest volume was in 1999 when the total had been 57,000 tonnes worth US$ CIF 10.6 million in 1992 dipped to 26,000 tonnes worth US$ CIF 7.5 million in 1999, representing a 10.6% negative growth rate. The country supplied 13.1% of total US imports in 1999. In contrast, exports to the European Union increased from 396 tonnes in 1995 to 546 tonnes in 1998, representing an 11.3% growth rate.

Venezuela is the third-largest supplier of plantains to the North American market with an average of 8.2% over the past eight years and a 13% contribution to US supplies in 1999 equalling that of Ecuador. The country has gradually increased its market share, with an increase from 16,000 tonnes in 1992 worth US$ CIF 6.5 million to 26,000 tonnes in 1999 worth US$ CIF 17.2 million, i.e. a 68% growth rate. In contrast, its shipments to the European Union decreased from 33 tonnes in 1994 to 12 tonnes in 1998, a 22.4% negative growth rate. Costa Rica and Colombia profited from this to increase their market shares.

International prices

The price of plantain has not in general increased significantly in the North American market in the past eight years. The Dominican Republic obtains the highest price at US$ 0.58 per kg, followed by Venezuela with US$ 0.45, Costa Rica and Colombia with US$ 0.39 and finally Ecuador with US$ 0.19.

Figure 1 shows that Venezuela holds the historic price record in the face of Colombia and Ecuador. This is because Venezuelan plantains are larger than fruits from Colombia and Ecuador and are much appreciated by the Latin American communities living in the United States and especially in Miami and New York, where most of the Latin Americans and West Indians who eat green plantain are to be found.

Plantain is more expensive on European markets than in North America. This is mainly the result of high transport costs and customs dues, without forgetting that plantain is an exotic fruit on this type of market. Figure 2 shows that the price of fresh plantain has varied between US 0.06 and 1.64 per kg. Another feature is that the highest price was obtained by an African country, Ghana, with an average of...
US$ 1.53 per kg over a 4-year period, followed by Dominica (Lesser Antilles) with US$ 0.99 per kg. Venezuela with US$ 0.75 per kg (the latter country displayed a 77.5% negative price trend from 1996 to 1998), Costa Rica at US$ 0.63 per kg and finally Colombia with an average of US$ 0.58 per kg. Prices were stable for the last two years of the period analyzed.

It should be noted that Colombian produce fetched higher prices than that of Costa Rica in France and Great Britain in 1998. The price in Great Britain varied between US$ 0.4 and 1.7 per kg. From February 1999 onwards, Colombian plantain fetched US$ 0.1 to 0.5 less than Costa Rican produce because of a decrease in supplies from the Uraba region. Prices on re-exported plantain markets are distinctly higher. Plantain is re-exported all the year round on the French and British markets and the best prices are obtained in Britain (CCI 1998, 2000).

Plantain growing at the national scale

Table 2 shows production distribution in 1999 according to the natural geographic zones. The Andean region is the most important production zone with 64% of the cultivated area producing 67% of total national production. Then, in descending order, come the Pacific region with 12% of the cultivated area and 9% of production, followed by 24% of production and cultivated area in the Caribbean regions, the Orinooco, Amazonia and the islands of San Andrés and Providencia.

The departments with the largest cultivated areas and production at national level are Antioquia, Quindío and Tolima with respectively 14%, 10% and 9% of the area under plantain. In production, Quindío and Antioquia account for 14% and Tolima 10%.

Plantain is grown in mixed cropping systems with coffee (81%), as a sole crop (15%) and intercropped 4%.

Types of producers

Four producer categories can be established (small, medium, large and industrial) (Table 3) on the basis of the number of hectares cultivated and the type of holding, with the main cropping system being mixed cropping followed to a lesser extent by sole cropping (Rodríguez Saavedra et al. 1999).

In all cases, production is marketed locally, nationally or internationally according to the volumes produced, with the exception of small producers who reserve the crop for personal consumption or for animal feed.

Industrial exploitations and sometimes the large producers use specialized technical assistance, whereas the majority of small and medium producers do not use this type of service (Rodríguez Saavedra et al. 1999).

National consumption

Plantain is a crop of great strategic importance in the rural sector in Colombia. It also has a privileged position in urban food distribution. Plantain is eaten both green and very ripe and is prepared using different recipes in the various regions of the country. It is also found in the form of meal, chips and snack foods but only a very small percentage of production is processed industrially.

Over the past eight years, fresh plantain consumption has decreased from 73.3 to 61.9 kg per person per year, i.e. negative growth of 2.4% from 1992 to 1999. In contrast, the per capita consumption of processed plantain increased by 6% over the same period (from 0.02 to 0.03 kg per person per year). This is explained by changes in eating habits, with the trend being towards processed products (CCI 2000).

It can be seen that the prospects for agri-industrial demand for the product are favourable. Indeed, consumption increased from 900 tonnes in 1992 to 2000 tonnes in 1999, a 12.1% growth rate. The processing industries consider that this trend may continue during the
coming five years if consumer interest in this type of product does not weaken (CCI 2000).

Figure 3 shows that Bogotá is the largest plantain consumer with 29% consisting of 70% Hartón and 30% miscellaneous clones such as Cachaco and Dominico hartón. Bogotá is followed by Medellín and Cali with 17% and 14% respectively, consisting of 80% Dominico hartón and 20% Hartón. Barranquilla comes last with 5% of national consumption, mainly Hartón. Nearly 20% of consumers at the Cali, Barranquilla and Bogotá markets and 32% of those in Medellín prefer ripe plantain (CCI 2000).

Job creation

The mechanized, traditional or intercrop cultivation cultivation of one hectare of plantain generates respectively 1.68, 0.39 and 0.19 permanent direct jobs per ha and per year. In the light of this, it is estimated that one hectare of plantain generates an average of 0.75 permanent job. When set against the national cultivated area, this gives approximately 288,375 permanent jobs. This is the equivalent of 58,000 families of five persons devoted to growing plantain.

National prices

Although plantain is a crop with permanent production, harvesting periods are influenced by external factors such as coffee production and harvesting or by very cold seasons. These production movements or periods in turn cause upward or downward price trends according to supply and demand volumes (Rodríguez Saavedra et al. 1999).

It should be noted that the three main wholesale markets (Bogotá, Cali and Medellín) display identical behaviour in both supply and demand, even though plantains are harvested continuously (Rodríguez Saavedra et al. 1999).

Seasonal variations in current prices on the three main wholesale markets from 1992 to 1999 are shown in Figure 4. It can be seen that these prices rise from January to April, with lower prices in Bogotá. In the second semester, prices fall in Cali and Medellín and remain very high in Bogotá until September. The situation is then reversed and prices finally fall on the three large wholesale markets in November-December.

A decrease in real incomes according to climatic features can be seen for both producers and resellers as a result of various factors including the effect of «El Niño», which disturbed the climate from March 1997 to the first half of 1998 and that of «La Niña» from the second half of 1998 until the first half of 1999. These phenomena had a direct effect on production volumes and resulted in smaller supplies and high prices.

Marketing

Marketing channels

The marketing of plantain is very difficult because of the dispersal of the production zones, the lack or poor conditions of the lines of communication with urban consumption centres and the irregular supplying of the market by wholesalers and middlemen who set the prices. In addition, perishable produce like plantain suffers from continuous spoilage and breaks down very quickly.

Table 2. Cultivated areas, production and plantain yield in 1999 in different regions of Colombia (Carlos Humberto Gutiérrez, Minagricultura, June 2000).

<table>
<thead>
<tr>
<th>Region</th>
<th>Cultivated area (ha)</th>
<th>Production (t/year)</th>
<th>Yield (t/ha/year)</th>
<th>% production</th>
<th>% cultivated area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caribbean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guajira</td>
<td>2 276</td>
<td>14 339</td>
<td>6.3</td>
<td>0.58</td>
<td>0.63</td>
</tr>
<tr>
<td>Magdalena</td>
<td>1 780</td>
<td>11 715</td>
<td>6.6</td>
<td>0.47</td>
<td>0.50</td>
</tr>
<tr>
<td>Cesar</td>
<td>3 381</td>
<td>23 905</td>
<td>7.1</td>
<td>0.97</td>
<td>0.94</td>
</tr>
<tr>
<td>Atlántico</td>
<td>418</td>
<td>3 201</td>
<td>7.7</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>Bolívar</td>
<td>5 417</td>
<td>35 980</td>
<td>6.6</td>
<td>1.46</td>
<td>1.51</td>
</tr>
<tr>
<td>Sucre</td>
<td>1 027</td>
<td>4 886</td>
<td>4.8</td>
<td>0.20</td>
<td>0.29</td>
</tr>
<tr>
<td>Córdoba</td>
<td>25 101</td>
<td>169 496</td>
<td>6.8</td>
<td>6.87</td>
<td>7.00</td>
</tr>
<tr>
<td>Sub-total</td>
<td>39 400</td>
<td>263 522</td>
<td>6.7</td>
<td>10.68</td>
<td>10.99</td>
</tr>
<tr>
<td>Pacific</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choco</td>
<td>16 245</td>
<td>98 541</td>
<td>6.1</td>
<td>3.99</td>
<td>4.53</td>
</tr>
<tr>
<td>Cauca</td>
<td>5 576</td>
<td>34 937</td>
<td>6.3</td>
<td>1.42</td>
<td>1.56</td>
</tr>
<tr>
<td>Narino</td>
<td>20 561</td>
<td>88 681</td>
<td>4.3</td>
<td>3.60</td>
<td>3.74</td>
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<tr>
<td>Sub-total</td>
<td>42 382</td>
<td>222 159</td>
<td>5.2</td>
<td>9.01</td>
<td>nd</td>
</tr>
<tr>
<td>Andean and inter-Andean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioquia</td>
<td>49 594</td>
<td>340 041</td>
<td>6.9</td>
<td>13.78</td>
<td>13.83</td>
</tr>
<tr>
<td>Valle del Cauca</td>
<td>11 985</td>
<td>127 283</td>
<td>10.6</td>
<td>5.16</td>
<td>3.34</td>
</tr>
<tr>
<td>Caúdas</td>
<td>18 651</td>
<td>106 675</td>
<td>5.7</td>
<td>4.32</td>
<td>5.20</td>
</tr>
<tr>
<td>Risaralda</td>
<td>18 135</td>
<td>72 227</td>
<td>4.0</td>
<td>2.93</td>
<td>5.06</td>
</tr>
<tr>
<td>Quindio</td>
<td>36 080</td>
<td>345 262</td>
<td>9.6</td>
<td>14.00</td>
<td>10.06</td>
</tr>
<tr>
<td>Tolima</td>
<td>32 972</td>
<td>234 581</td>
<td>7.1</td>
<td>9.51</td>
<td>9.20</td>
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<tr>
<td>Cundinamarca</td>
<td>12 808</td>
<td>127 932</td>
<td>10.0</td>
<td>5.19</td>
<td>3.57</td>
</tr>
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<td>Boyaca</td>
<td>3 305</td>
<td>39 413</td>
<td>11.9</td>
<td>1.60</td>
<td>0.92</td>
</tr>
<tr>
<td>Santander</td>
<td>8 530</td>
<td>70 842</td>
<td>8.3</td>
<td>2.87</td>
<td>2.38</td>
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<td>Norte Santander</td>
<td>12 475</td>
<td>89 223</td>
<td>7.2</td>
<td>3.62</td>
<td>3.48</td>
</tr>
<tr>
<td>Huila</td>
<td>26 638</td>
<td>95 310</td>
<td>3.6</td>
<td>3.86</td>
<td>7.43</td>
</tr>
<tr>
<td>Sub-total</td>
<td>231 173</td>
<td>1 648 789</td>
<td>7.1</td>
<td>66.84</td>
<td>64.48</td>
</tr>
<tr>
<td>Orinooco</td>
<td></td>
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<tr>
<td>Arauca</td>
<td>8 909</td>
<td>60 976</td>
<td>6.8</td>
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<td>2.49</td>
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<td>Casanare</td>
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<td>8.2</td>
<td>0.79</td>
<td>0.66</td>
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<td>Vichada</td>
<td>157</td>
<td>1 413</td>
<td>9.0</td>
<td>0.06</td>
<td>0.04</td>
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<td>Meta</td>
<td>11 458</td>
<td>117 881</td>
<td>10.3</td>
<td>4.78</td>
<td>3.20</td>
</tr>
<tr>
<td>Sub-total</td>
<td>22 891</td>
<td>199 709</td>
<td>8.7</td>
<td>8.10</td>
<td>6.39</td>
</tr>
<tr>
<td>Amazonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amazonas</td>
<td>243</td>
<td>413</td>
<td>1.7</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Caquetá</td>
<td>10 094</td>
<td>61 629</td>
<td>6.1</td>
<td>2.50</td>
<td>2.82</td>
</tr>
<tr>
<td>Guainia</td>
<td>547</td>
<td>3 702</td>
<td>6.8</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Guaviare</td>
<td>4 252</td>
<td>21 718</td>
<td>5.1</td>
<td>0.88</td>
<td>1.19</td>
</tr>
<tr>
<td>Putumayo</td>
<td>7 033</td>
<td>41 333</td>
<td>5.9</td>
<td>1.68</td>
<td>1.96</td>
</tr>
<tr>
<td>Vaupés</td>
<td>476</td>
<td>3 630</td>
<td>7.6</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Sub-total</td>
<td>22 645</td>
<td>132 425</td>
<td>5.8</td>
<td>5.37</td>
<td>6.32</td>
</tr>
<tr>
<td>San Andrés y Prov.</td>
<td>14 152</td>
<td>10.9</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>TOTAL</td>
<td>358 505</td>
<td>2 466 756</td>
<td>6.9</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Table 3. Types of producers, holding size and cropping system (Rodríguez Saavedra et al. 1999).

<table>
<thead>
<tr>
<th>Type of producer</th>
<th>Holding size (ha)</th>
<th>Cropping system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>0.1-5.0</td>
<td>Intercropping**</td>
</tr>
<tr>
<td>Medium</td>
<td>5.1-15.0</td>
<td>Mixed**</td>
</tr>
<tr>
<td>Large</td>
<td>15.1-30.0</td>
<td>Mixed</td>
</tr>
<tr>
<td>Industrial</td>
<td>&gt; 30.1</td>
<td>Mixed</td>
</tr>
</tbody>
</table>

*Without uniform spatial distribution and that may include various cultivated plant species
**Distribution is according to planting systems defined in relation to the main crop in the mixed system.
Figure 3. Distribution of plantain consumption in Colombia (CCI 2000).

Figure 4. Index of seasonal variations in plantain prices at the three main wholesale markets in Colombia, 1992-1999 (Calculation by Corpoica Regional Nueve, Oficina de Planeación, using Cordo Café and CCI data for 1992-1999).

deterioration resulting from poor post-harvest management. This aggravates loss of quality and quantity and thus affects the final price (Rodríguez Saavedra et al. 1999).

As plantain is a fruit that is generally eaten fresh and marketing is immediate, it has the marketing characteristics specific to all perishable foodstuffs whose production is complex and whose distribution is difficult to organize rationally. The process involves a large number of producers and a few wholesalers who distribute plantain to consumers on a large scale. As the wholesalers are not numerous, information about plantain moves between them rapidly, enabling them to agree, among other things, about prices and the quantities to be put on the market (Rodríguez Saavedra et al. 1999, CCI 2000).

Most plantain producers are small growers who are very scattered and generally sell the fruit at the farm gate. Middlemen therefore play an essential role in the coordination of purchases of plantain, transport and sale, thus being able to pocket a larger proportion of the value added during the process (Rodríguez Saavedra et al. 1999).

The traditional markets consisting of purchase centres, permanent and temporary markets, a few supermarkets and shops are characterized by control by middlemen. In order to define the conditions of negotiation—given the heterogeneity of the fruits—all the plantains must be presented at the site of the transaction (Rodríguez Saavedra et al. 1999).

The specialized market is characterized by an appropriate organization structure for selection, sorting and packing. Supermarket chains accept or not the batches presented by suppliers on condition that they have seen a sample of the produce, that their quality criteria are met and the supplies guaranteed. A price range is generally set in this type of transaction in order to avoid fluctuations that are too sudden and require produce classification in conformity with the types usually sold (Rodríguez Saavedra et al. 1999).

As everywhere, the national plantain market responds to the requirements of supply and demand but lacks a regulating body, which has contributed to the development of complex marketing channels. In this context, five main channels leading to the consumer can be identified: collector>wholesaler>retailer; supplier>wholesaler>supermarket; producer>supermarket; wholesaler>agri-industry; and producer>agri-industry (Rodríguez Saavedra et al. 1999, CCI 2000).

On-farm post-harvest losses
Post-harvest fruit losses are estimated to be 10% On the basis of national production of 2.5 million tonnes in 1999, fruit losses are estimated at 250 000 tonnes, representing 62.5 thousand million Colombian pesos (COP) (US$ 36 million) assuming an average farm gate price of COP 250 per kg (1 US dollar = 1758.11 Colombian pesos in 1999). These figures clearly show the need to develop a process that prevents economic losses and also generates value added for fresh plantain and avoids problems of pollution resulting from the poor use of crop residues.

The causes of losses consist mainly of the poor level of technology at cropping level, poor harvesting, inefficient handling of produce from production to consumption sites and poor product conformity. Packing and especially transport affect quality and the appearance of fruits since middlemen pay no attention whatsoever to the improvement of the packing system for fruit transport. Bunches are generally transported in bulk, resulting in bruising and
scarring and hence poor appearance and decreased quality (Roldríguez Saavedra et al. 1999).

For specialized markets, the produce is packed and shipped in crates that protect the fruits during distribution operations and result in better product acceptance by the consumer (Roldríguez Saavedra et al. 1999).

Agri-industrial development

Hartón and Dominico hartón clones cultivated in hot zones are easier to peel and thus potentially easier to process. The processing industries have set distinctions between the two clones. Hartón fruits are larger and have a higher moisture content; Dominico hartón fruits have a higher soluble matter content. It should be specified that no conclusive results allow such characterization of the clones and their advantages for processing (CCI 2000).

In Colombia, the preference is for fresh fruits and the taste for meal or chips is minor. The agri-industrial development of plantain processing in the central coffee growing zone is recent. An agri-food plant was established in early 2000 at Murrillo, in the Armenia municipal district, where ‘patacones’ (deep-fried crushed plantain portions) and slices frozen for distribution in supermarkets are prepared. Plantain is also packaged whole and frozen for certain factories that export the fruit in snack form, as meal or frozen for international markets.

According to Day (1987), the pseudostem, leaves, flowers and roots can be used after the harvest for the production of meal, vinegar, paper, edible tortas, chipboard, animal feed, dyes, etc.

Expectations for future agri-industrial development are great in the central coffee-growing region.

National and international market opportunities for plantain

Colombia could increase plantain exports to North America, especially in the form of snack foods and baby food if the consumption of fresh and processed plantain increases in Latin American, African, Anglo-Saxon and European groups (CCI 2000).

 Ministry of Agriculture forecasts for 2000 indicate that production will not cover domestic market demand, even though fresh consumption has decreased. This requires new areas for the crop or establishment of technology transfer to turn some holdings into intensive, mechanized operations to meet unsatisfied demand. This would prevent the increasing volume of plantain imports from Ecuador and Venezuela (CCI 2000).

References


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E-mail: corpoica@col2.telecom.com.co

The production of fire-softened plantain leaves for the agrifood industry

E. Echeverry Navarro

The flat, hot zone in the central southern part of Tolima department is inhabited almost entirely by descendants of the “Pijao” tribe. Many of them, grouped in native councils, work in farming and small-scale animal husbandry.

One of the main crops in their subsistence farming practices is the ‘Cachaco común’ plantain clone (Musa ABB, Simmonds) that they use to produce leaves to be fire-softened for the agrifood industry. The clone has displayed very good adaptability at an elevation of 400 m to difficult soil and climate conditions, characterized by degraded, low-fertility soils, a hot, dry climate, annual precipitation of 1000 to 1300 mm poorly distributed over the year and an average annual temperature of 25°C.

Within the zone studied (600 ha), 4500 to 5000 persons participate in the production of fire-softened leaves and live on the proceeds of their sale in bundles of 50. The production process
is handled entirely by family groups consisting of fathers and their sons, whatever their age.

The leaves of 'Cachaco común' are those most frequently used to wrap cooked foods because they do not cause any modifications to the organoleptic properties of foods and are fully sterilized by exposure to flame to soften them. This is not the case of leaves of plantains of other clones such as 'Harton' for example, which, among other things, gives tamales\(^1\) and cheeses a greenish color when used to wrap them. Tamales have a particularly pleasant smell when they are wrapped in 'Cachaco común' plantain leaf.

No work previous to this is known on plantations of 'Cachaco común' plantain devoted exclusively to leaf production for the agroindustry. This explains why plant reactions to frequent, severe defoliation are not known. It can be expected that the plant would increase its leaf emission rate and number, as reported by Belalcázar (1991), unless, in contrast, the number decreases, but this is less likely.

When a small grower of 'Cachaco común' plantain decides to produce leaves, we know in advance that he prefers to sell leaves every week or every fortnight rather than the fruits. In addition, any fruit bunches produced under these conditions are very small with poorly filled fingers for lack of leaves.

Today, a bundle of 50 fire-softened plantain leaves fetches 2000 to 2500 Colombian pesos (COP), i.e. US$ 1 to 1.25, roughly the equivalent of a bunch of plantains. In a year, a plant produces 150 to 175 leaves worth 6000 to 7500 pesos against only a single marketable bunch of 'Cachaco' plantains worth hardly 2500 pesos.

Leaf production is continuous and steady – and can even increase – in subsequent years in the same plantation, whereas fruit bunch production decreases in both quality and quantity after the first production cycle.

Review of the literature
According to the studies performed by Martínez (1984), Arévalo (1986) and Belalcázar (1991), plantain can keep up to 16 functional, erect, green, healthy leaves. This corresponds to a growth cycle of about 120 to 130 days per leaf when the agro-meteorological conditions (mainly soil, precipitation, temperature, wind and relative humidity) are favourable for plant development and if there are no diseases – especially leaf diseases. Up to now, defoliation or deleafing of plantain has been for phytosanitary reasons and consists of removing all the infected leaves or those that have withered to more than 60%.

In general, the leaves of adult plantain are 70 à 100 cm wide and 150 to 400 cm long, with leaf indexes varying from 2 to 4 according to the clone and soil and climate conditions. Leaf thickness varies from 0.35 to 1 mm according to the portion of lamina considered and the polyplody (Champion 1978, Belalcázar 1991).

Under good conditions and in its environment, plantain emits a leaf every 8 to 10 days. In addition, the obtaining of a good bunch of plantain requires at least 7 to 8 functional leaves at flowering (male flower emission) (Arcila et al. 1994, Belalcázar et al. 1996, Martínez 1984).

It has been shown in other studies that plantain requires 8 functional leaves for bunch size and weight not to be reduced (Martínez 1984). Bunch weight is reduced by 50 and 40% respectively when there are only 4 and 6 functional leaves during the vegetative cycle.

In addition, Belalcázar (1991) also stressed that cutting the green leaves in addition to wilted leaves before flowering leads to certain benefits, including:
- a strengthening of the plant physiological processes leading to an increase in production;
- improved penetration by light to the foot of the plant, stimulating budding and sucker development;
- enhanced aeration, reducing relative humidity that is dangerously suitable for the development of diseases;
- more rapid decomposition of organic material;
- a decrease in water loss by transpiration during drought periods.

Research conducted by Belalcázar (1991), Martínez (1984) and Merchán (1994) among others confirmed that plantain emits from some 36 to 39 leaves during the vegetative period, except under extreme climatic conditions that are difficult to handle.

Belalcázar et al. (1998) consider that of all triploid plantains with balbisiana dominance (ABB), 'Cachaco común' would appear to be the hardest and most tolerant of drought and water stress.

Material
The study was conducted for 15 months from November 1996 to January 1998 on a farm at Agua Fría in Coyaíma, in the central southern part of Tolima department (Colombia). The farm is at an elevation of 400 m. Annual precipitation is from 1000 to 1300 mm with irregular bimodal distribution. Annual average temperature is 25°C.

1997 was climatically atypical. Rainfall was generally very scarce throughout the year as a result of the Pacific meteorological phenomenon 'El Niño'. This drought caused the disappearance of numerous small plantain plantations and, from time to time, sharp price rises for leaves from COP1500 to 3500 per bundle, a price considered very high by middlemen and wholesalers but judged to be extremely satisfactory by the farmers.

The farm at which the study was conducted has a loamy-sandy soil. With a pH of 6.9, the soil has a small organic percentage (1.3%), an average sulphur level (6.0 ppm), large quantities of phosphorus (42.9 ppm), copper (1.13 ppm), iron (18.4 ppm) and manganese (37.02 ppm), small quantities of zinc (0.72 ppm) and boron (0.19 ppm), high calcium (18.43 meq/100 g soil) and magnesium levels (4.03 meq/100 g soil), a low potassium content (0.15 meq/100 g soil) and a normal sodium content (0.10 meq/100 g soil).

'Cachaco común' plantain was used. This has the best behaviour in the zone thanks to its hardiness and drought tolerance. It is also the clone most commonly used for the production of fire-softened leaves for the agroindustry, used for tamales, cheeses, en vueltos\(^2\) made with plantain, maize, rice, etc. No fertilizer or pesticide of any kind was used.

Methodology
A total of 96 suckers were planted in 30 cm x 30 cm x 30 cm holes with spacing of 2 m x 2 m, in conformity with the practices in the region.

Soil analysis was performed but was not followed by fertilization. In a previous experiment, the application of inorganic fertilizers such as nitrogen (urea 40% N) and potassium (potassium chloride 60% K2O) had caused the total blackening of leaves when exposed to flame and they could therefore not be used in the agroindustry.

Weeding was performed by hand three times during the experiment (15 months). Desuckering was not performed. Axillary buds were left to grow around each mother plant as is the custom in the region as the more plants per mut, the more leaves can be harvested.

The treatment was fully random with four protocols with three repetitions in

\(^1\) Tamales are a typical Colombian dish made with chicken, vegetables and maize flour steamed in plantain leaf.

\(^2\) En vueltos are flat cakes made with maize, potato or plantain and wrapped in plantain leaf.
each case. The protocols consisted of leaving 3, 4, 5 or 6 leaves on all the plants in a mat after each harvest or leaf cutting operation as shown in Table 1.

Each protocol was applied to a row of eight plants or eight mats each consisting of a mother plant and its suckers.

The first three cuts were performed at 3-week intervals. Then, with no rainfall, cutting was performed every 4 weeks until a total of 8 leaf harvests had been reached, considered to be a low figure for a 14-month period. A leaf forms every 8 days under normal rainfall conditions and every 10 to 12 days during drought periods.

Results and discussion

Growth parameters
Table 2 presents the growth measurements performed during the eight leaf harvests of the experiment.

The average values of the growth parameters (height and girth) of 'Cachaco común' plantain at Coyaima in Tolima department (Table 2) do not display statistically significant differences, suggesting that different levels of systematic defoliation do not affect the vegetative development of the plants.

Leaf production parameters
The two parameters measured immediately after leaf harvesting and which are used as indicators of the biomass produced in each protocol are shown in Table 3.

The figures for total marketable leaves harvested during the eight harvests performed during the experiment clearly show that the best treatment was protocol No. 1, followed by Nos. 2 and 3, which yielded respectively 72.6% and 65.2% of the amount in No. 1. No. 4 gave the smallest number of leaves at only 42.4% of the amount in No. 1.

Leaf weight, length and width
The leaf weight parameter is neither decisive nor essential for leaf production for the agrifood industry since it does not determine the price to be paid. The most important criteria are length and width and above all the sanitary condition of the leaves.

Leaf length and width are not very precise characteristics. A 'Cachaco común' plantain leaf is considered to be suitable for packing or containing tamales, cheeses, envueltos and other food products when the lamina is more than 1 metre long and 30 cm wide in the central portion. These two parameters were measured during the experiment each time that leaves were cut or harvested, but for reference purposes only. It is noted that the longest leaf was observed in protocol No. 2 at 206 cm long, not counting the petiole, and 69 cm wide in the central part, followed by a leaf 196 cm long and 67 cm wide in protocol No. 4.

Cutting period
Unless there are diseases or serious wind damage, the moment for cutting 'Cachaco común' leaves depends mainly on the frequency of rainfall and market conditions.

Under the climatic conditions at Coyaima, plants emit a leaf every 7 or 8 days during the rainy season and every 10 to 11 days during the dry season. Cutting is performed when one, two or three leaves are ready for fire-soften or, more specifically, when there is market demand. However, the general custom is that of harvesting every fortnight in the rainy season and every three weeks in the dry season.

Economic parameters
After cutting, the leaves are exposed to a flame for softening on the spot. They are then folded and assembled in tied bundles of 50 leaves. The bundles are sold directly to middlemen/transporters who pay COP2000 to 2500 (US$1 to 1.25) depending on the time of year and according to supply and demand.

Purchases are completed on a cash basis at mobile buying points at different places in the production zone and on different days according to the time of year, and especially on the occasion of the mid-year and end-of-year festivities.

Leaves thus fetch the best prices during the St John's day and St Peter's day festivities in June and then in December and January over Christmas, the New Year and Epiphany. The price of leaves falls a little during the rainy season because production is substantial and supplies are plentiful. It also decreases during the months in which demand is weak.

Conclusions
- Most of the saleable fire-softened 'Cachaco común' plantain leaves were obtained when at least three leaves per plant were left after the cutting of leaves for sale. This was performed without desuckering or fertilizer or pesticide application.

- Leaf production was largest with protocol No. 1 in which three leaves were left on each plant after cutting. The
smallest production was given with protocol No. 4, in which six leaves were left on each plant.

- The largest number of leaves in a single cut was 150 in protocol No. 1.

- Leaves are sold at mobile installations in the production areas on days set beforehand with wholesalers who compete with each other.

- Leaves are in bundles of 50 sold directly for cash to midmen/transports at prices ranging from COP 1500 to 2500 per bundle.

- Producer prices are highest in June, December and the first fortnight in January. This coincides with the festivities at those dates, when demand for cooked food, and especially *tamales tolimenses*, is at its highest.

- The time for cutting leaves depends mainly on the time of year (rainy season or dry season). Thus, leaves can be cut every fortnight or even every week during the rainy season but the interval is three weeks in the dry season and may even be four weeks in the most extreme cases.

**Acknowledgements**

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**References**


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**Agronomy Rapid propagation**

**The evolution of photosynthesis, transpiration and chlorophyll during the development of leaves of plantain (*Musa* AAB Simmonds)

G. Cayón S.

The growth and development of a cultivated plant depends essentially on the progressive increase of its leaf area, which enables it to make more effective use of solar energy during photosynthesis. The interception of solar radiation by the leaf surface is influenced by the size, shape, age, angle of insertion on the stem, vertical separation and horizontal position of the leaf (Yoshida 1972). The angle of insertion is very important for crop productivity as this governs the exposure of the leaves to sunlight and hence the more even distribution of light through the plant canopy, enabling more efficient photosynthetic activity at the mid and lower levels of the plant (Cayón 1992). The chlorophyll present in all green plants is one of the pigments most closely related to photosynthetic efficiency, plant growth and environmental adaptation. Kumar et al. (1972) demonstrated the existence of a chlorophyll gradient in sugar cane running from the top to the base of individual leaves and also between leaves of different ages. Photosynthesis varies greatly according to the age of the plant. As a leaf develops and the chloroplasts become organized, photosynthetic activity increases rapidly to a peak level attained when growth of the lamina is complete and...
then gradually decreases during leaf ageing.

It was long believed that the rates of photosynthesis of perennial crops were lower than those of herbaceous plants, but recent research has demonstrated that many trees and shrubs, including certain conifers, display maximum photosynthetic rates that are very close to those of C₃ plants (Catsky et al. 1987). The highest photosynthetic rate is attained in most leaves when the lamina has fully unrolled; the rate then decreases with age. This decrease in photosynthetic capacity is typical of leaves of perennial and short-cycle plants (Silveira 1987).

The aim of this work was to determine the behaviour and intensity of gas exchange and of the chlorophyll synthesis and degradation processes during the development of plantain leaves.

**Material and methods**

The experiment was conducted at the Palmira Research centre in the Valle del Cauca department, at 3°31'N and 76°19'W, elevation 1001 m asl, average annual temperature 24°C, average relative humidity 75% and annual precipitation 1000 mm. These climatic conditions are those of dry tropical forest (dfT). The experimental plot had clay loam soil at pH 6.8 and contained 2.9% organic matter. The clone Dominico horton was planted at 3.0 m between rows and 2.0 m between plants along the rows with one sucker per planting hole and overall density of 1666 plants ha⁻¹. A fully randomized experimental protocol was used with three repetitions and six plants per repetition.

When the plants had emitted 16 leaves (five months after planting), the youngest fully unrolled leaf (leaf 1) was identified on each one and the net photosynthetic rate, transpiration and chlorophyll were measured every 20 days from complete unrolling of the half-lamina (day 0) to total leaf senescence (day 140). The photosynthetic and transpiration rates were measured in the central part of the leaf using an LI-6200 portable photosynthesis system (Licor). Chlorophyll was measured using the ethanol extraction method (Wintemans et al. 1965) on 1.3 cm² leaf discs taken from the same central leaf section as that used for the measurement of gas exchanges. Extraction was performed by cold maceration of each leaf disc in a mortar containing 4.0 ml of a solution (ethanol 98% + MgCO₃, 0.5 g l⁻¹), transfer of the extract to a test tube and washing the mortar with 4.0 ml solution to complete the final volume to 8.0 ml. The extract was separated by centrifugation at 3000 x g for 5 minutes. Absorption at 649 and 665 nm were then read using a Spectronic 21 spectrophotometer and the data were used to calculate chlorophyll a (Cₐ), b (C₋b) and total chlorophyll (Cₐt), using the following formulas:

\[ Cₐ = [(137.8 x A₃₆₅) - (57.6 x A₆₄₉)] x V/DW \]

\[ C₋b = [(258 x A₆₄₉) - (76 x A₆₆₅)] x V/DW \]

\[ V = \text{final volume of ethanol extract (8.0 ml)} \]

\[ DW = \text{leaf disc dry weight (g)} \]

The results were subjected to analyses of variance, correlation and regression using the MSTATC statistical program (Michigan State University).

**Results and discussion**

It is seen in Figure 1 that the evolution of gas exchange rates (photosynthesis and transpiration) and that of chlorophyll synthesis follow a quadratic regression pattern during leaf development.
Table 1. Correlation matrix for leaf age, photosynthesis, transpiration and total chlorophyll concentration.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Leaf age</th>
<th>Photosynthesis</th>
<th>Transpiration</th>
<th>Total chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf age</td>
<td>1.00</td>
<td>-0.783**</td>
<td>-0.668**</td>
<td>-0.634**</td>
</tr>
<tr>
<td>Photosynthesis</td>
<td>-</td>
<td>1.000</td>
<td>0.771**</td>
<td>0.842**</td>
</tr>
<tr>
<td>Transpiration</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>0.538*</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
</tr>
</tbody>
</table>

** significant (P<0.01) * significant (P<0.05).

Life from day 0 to day 140. The photosynthetic rate is low during the initial leaf development stage and increases rapidly to maximum (12.22 μmol CO₂ m⁻² s⁻¹) attained 20 days after leaf unrolling (DAU); it then decreases slightly, remaining fairly steady until 80 DAU, and finally decreases considerably until the death of the leaf blades (140 DAU). The lower leaf photosynthetic level from the youngest stage (0 DAU) is explained by the fact that the photosynthetic and enzymatic systems are not fully formed. Chlorophyll synthesis is in the initial stages, pigment concentration is not sufficient to capture the solar energy required for photosynthesis and the stomata have not gained their full physiological capacity. It is sufficient to observe the pale green colour after the unrolling of the 'cigar'. The maximum photosynthetic rate (12.2 μmol CO₂ m⁻² s⁻¹) of each newly formed leaf is conserved for a comparatively short period of time (20 days), after which it decreases slightly and remains at between 5.53 and 7.12 μmol CO₂ m⁻² s⁻¹ for 60 days before reaching a minimum at total senescence (140 DAU). The drastic decrease in the photosynthetic rate during leaf senescence results in a negative carbon balance since leaf respiration is constant throughout the plant development process.

Leaf transpiration is also low at the beginning of development and then increases to a maximum on 50 DAU and finally decreases as the leaf ages. It can be seen that leaf transpiration continues until 60 DAU, that is to say for a longer period than photosynthesis and this probably accelerates ageing. Plantain is phyllotaxic and continuously emits new leaves that change position during plant development and hence change their exposition to sunlight, and eventually become partially shaded. This contributes to the gradual decrease in photosynthetic and transpiration rates, with repercussions on gas exchanges in the plant. These results are in agreement with those of different authors who have studied interactions between the age and physiological activity of banana and plantain leaves during the vegetative growth phase. They observed the highest photosynthetic and transpiration rates in the youngest leaves (leaves 2, 3, 4 and 5) and drastic decreases in the oldest leaves (leaves 6, 7, 8 and 9) (Robinson and Bower 1988, Kallarackal et al. 1990, Eckstein and Robinson 1995, Cayón et al. 1998).

The evolution of the chlorophyll concentration is similar to that of photosynthesis and transpiration; maximum pigment concentrations are observed between 20 and 40 DAU and the level then decreases to minimum at complete senescence. The chlorophyll concentration is low during the leaf unrolling period because the leaf is not fully exposed to sunlight, and this governs the synthesis and accumulation of chlorophyll. The chlorophyll level increases markedly when leaf unrolling is complete and then remains constant during the intermediate period of leaf life before decreasing at the onset of senescence.

The photosynthetic and chlorophyll concentration rates are proportional during leaf development, with peaks on 20 DAU when the concentration of pigment for photosynthesis appears to be optimal. The photosynthesis process decreases considerably with a limiting chlorophyll concentration. On this subject, Cayón et al. (1994) observed that the maximum plantain leaf photosynthetic rate depended on the chlorophyll content and that the highest chlorophyll concentration was found in the central part of the leaf blade. Although loss of chlorophyll is a typical symptom observed during leaf senescence, its disappearance is nonetheless lower than that of the other photosynthetic compounds (Friedrich and Höfftaker 1980, Holloway et al. 1983, Kura-Hotta et al. 1987, Makino et al. 1983).

Studies conducted to explain the photosynthesis reduction mechanism during leaf senescence show that the phenomenon is caused by changes in the concentration and kinetics of the Rubisco enzyme (Evans 1986, Makino et al. 1985). The activity of the electron transport chain, directly correlated with photosynthesis activity, also decreases during leaf senescence, indicating that the decrease in photosynthesis is caused mainly by the functional degradation of the photosynthetic systems (Camp et al. 1982, Holloway et al. 1983, Kura-Hotta et al. 1987). The lower concentrations of chlorophyll and other active photosynthetic pigments can limit the leaf photosynthetic process. This reduces photosynthesis if the concentration falls below the optimum threshold (Gabrielsen 1948).

Photosynthesis, transpiration and chlorophyll concentration are inversely correlated with leaf age (P<0.001), showing that they depend on the ontogenesis of the leaf and decrease as the latter advances (Table 1). Photosynthesis is directly correlated with transpiration and chlorophyll content at all leaf development stages, showing that photosynthesis is functionally related to transpiration and depends on the chlorophyll concentration in the leaf blade.

The leaf insertion angle is an important parameter for plantain productivity as this governs the exposure of the leaves to sunlight and the distribution of photosynthetically active radiation (PAR) in the plant. Photosynthesis takes place in the various superposed leaf strata that shade each other in such a way that the incident PAR is absorbed through the strata, with a maximum in the best-exposed leaves. Photosynthesis is thus greater in leaves in the medium stratum; the lower leaves receive less PAR and have lower photosynthesis rates. Each leaf emitted changes position during plant growth, which means that its photosynthetic activity will only be at maximum while it is well exposed to PAR. As the results of this study show that the minimum photosynthetic rate of young leaves is conserved for a relatively short period of time (20 days) and that the rate then decreases considerably when they are shaded by new leaves, these new young leaves probably display physiological compensation by attaining their highest photosynthetic rate immediately after the preceding leaves. In addition, the fact that photosynthesis stabilizes at between 5.53 and 7.12 μmol CO₂ m⁻² s⁻¹ for the subsequent 60 days of development may be an essential contribution to the physiological processes of the plant. Indeed, from the strict point of view of production, it is very important that the functional leaves should maintain a moderate and constant photosynthetic activity over as long a period as possible.
Estimation of root development from shoot traits in plantain and banana (Musa spp.)

G. Blomme, R. Svennen, A. Tenkouano, R. Ortiz and D. Vuylstee

The root system is the link between the plant and the soil. It is responsible for the absorption of water and nutrients, anchorage, synthesis of some plant hormones and storage (De Langhe et al. 1983, Martin Prévèl 1987, Stover and Simmonds 1987, Lahav and Turner 1989, Price 1995). Root system development and shoot growth are highly related (Pearsall 1927, Broschat 1998, Fort and Shaw 1998). Russell (1977) mentioned that nodal root development in winter wheat and pearl millet could be estimated from the number of leaves. Henderson et al. (1983) found that the extent of coarse root branching was very regular for Sitka spruce and could be estimated using the aboveground stem diameter. Smith (1964) reported that root spread of several tree species could be estimated from aboveground measurements. In the case of banana, Svennen (1984), and Blomme and Ortiz (1996) observed positive correlations between root system development and aerial growth characteristics, while Gousseland (1983) estimated the number of cord roots of the ‘Giants Cavendish’ dessert banana from the leaf area. The objective of this study was to develop a method for estimation of root development from shoot characteristics, across a wide range of Musa genotypes.

Materials and methods
This study was carried out at the IITA High Rainfall station at Omne in southeastern Nigeria. Its soil is an ultisol derived from coastal sediments, well drained but poor in nutrients and with a pH of 4.3 in 1:1 H2O. The average annual rainfall is 2400 mm distributed monomodally from February to November. Details of the site were described by Ortiz et al. (1997). Twenty-seven genotypes representing the various Musa genomic and ploidy groups were assessed in this study. The planting material was obtained through meritism culture using the methods of Vuylstee
Table 1. Correlation coefficients (P<0.05) between aerial growth and root system characteristics at 20 WAP (weeks after planting)

<table>
<thead>
<tr>
<th>Trait</th>
<th>LA</th>
<th>PH</th>
<th>PC</th>
<th>LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR</td>
<td>0.72***</td>
<td>0.65***</td>
<td>0.65***</td>
<td>-0.09</td>
</tr>
<tr>
<td>NR</td>
<td>0.46*</td>
<td>0.41*</td>
<td>0.29</td>
<td>0.16</td>
</tr>
<tr>
<td>LR</td>
<td>0.64***</td>
<td>0.54**</td>
<td>0.46*</td>
<td>0.08</td>
</tr>
<tr>
<td>AD</td>
<td>0.47*</td>
<td>0.51**</td>
<td>0.70***</td>
<td>-0.38*</td>
</tr>
<tr>
<td>TL</td>
<td>0.41*</td>
<td>0.25</td>
<td>0.01</td>
<td>0.49*</td>
</tr>
<tr>
<td>TD</td>
<td>0.65***</td>
<td>0.53**</td>
<td>0.38</td>
<td>0.25</td>
</tr>
</tbody>
</table>

LA: leaf area (cm²), PH: height of the plant (cm), PC: plant circumference (cm), LS: length of the tallest sucker (cm), DR: root dry weight (g), NR: number of cord roots, LR: cord root length (cm), AD: average diameter at the base of the cord roots (mm), TL: total length of the cord roots of the mat (cm), TD: total dry weight of the roots of the mat (g).

*, **, *** Significant at P<0.05, 0.01 and 0.001, respectively.

Table 2. Regression models to predict root system characteristics at 20 WAP using aerial growth characteristics and ploidy level as independent variables.

<table>
<thead>
<tr>
<th>Trait</th>
<th>LA*</th>
<th>PC</th>
<th>LS</th>
<th>PL</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>B+10-DR</td>
<td>0.001628***</td>
<td>0.596934**</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td>0.00459***</td>
<td>1.255633***</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>0.006704***</td>
<td>23.476717***</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>0.009835***</td>
<td>0.681434***</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TL</td>
<td>0.099478***</td>
<td>14.69139***</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>0.002066***</td>
<td>0.426590</td>
<td>0.171415*</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

*^ independent variables.
* ** *** Significant at P<0.05, 0.01 and 0.001, respectively.

(1989, 1998). The plantlets were acclimatized for six weeks in a greenhouse nursery (Vuyasteke and Talengere 1998, Vuyasteke 1998), prior to transplantation in the field in June 1996. The experimental layout was a randomized complete block design with two replications of two plants per genotype.

The trial sites, which had been under grass fallow for eight years, were manually prepared with minimum soil disturbance. Planting was done using a spacing of 2 m x 2 m to avoid overlapping of adjacent root systems. The experimental area was treated with the nematocide Nemacur (a.i. fenamiphos) at a rate of 15 g per plant (three treatments year⁻¹) to reduce the nematode infestation. Plants were fertilized at 300 N and 450 K (kg·ha⁻¹·year⁻¹) split over six equal applications during the rainy season (i.e. February-November). The fungicide Bayfidal (a.i. triadimenol) was applied three times per year at a rate of 3.6 ml per plant to control the leaf spot disease black Sigatoka (Mycosphaerella fijiensis Morelet).

Shoot and root traits were assessed during the mid-vegetative growth (i.e. 20 week-old plants). Shoot growth characteristics included plant height (PH, cm), circumference of the pseudostem at soil level (PC, cm) and leaf area (LA, cm²). Leaf length and leaf widest width were measured and LA was calculated according to Obiefun and Ndubizu (1979). In addition, length of the tallest sucker (LS, cm) was measured. The root system was completely dug out and number of adventitious roots or cord roots (NR), average diameter at the base of the cord roots (AD, mm), dry weight of the roots (DR, g), length of the cord roots (LR, cm), total dry weight of the whole mat (i.e. plant crop and suckers) root system (TD, g) and total length of the cord roots of the whole mat (TL, cm) were measured. The average diameter of the cord roots was measured with a Vernier calliper, while the length of the cord roots was estimated according to the method of Newman (1966) and Tennant (1975).

Statistical analysis was carried out on the data set comprising 27 genotypes, using the SAS statistical package (SAS 1989). Relationships between aerial growth and root system characteristics were evaluated using correlation analysis. In addition, multiple regression analysis using stepwise selection was carried out. The dependent variables, i.e. the root system characteristics, were regressed on shoot growth characteristics and ploidy level (PL). Both correlation and regression analysis were carried out on 27 genotypes.

Results and discussion

Significant correlations between aerial growth and root system characteristics were found during the vegetative development (Table 1) and confirmed earlier reports (Beugnon and Champion 1966, Gousseland 1983, Swennen 1984, Lavi- gne 1987, Blomme and Ortiz 1996).

Regression analysis produced several equations, which attributed at least 90% of the variation in root growth to variation in shoot development. The best shoot indicators of root growth were leaf area, pseudostem circumference and length of the tallest sucker (Table 2).

These results suggest that reduced leaf area, as may be caused by black Sigatoka, will adversely affect root development. Conversely, increased leaf area as a result of fertilizer application would stimulate root development. The pseudostem is made up of leaf sheathes and hence reflects the number of leaves and plant vigour. Plant pseudostem circumference will thus reflect shoot growth and is an important determinant of root vigour in the regression models. The size of the tallest sucker contributed positively to the extent of the mat root system. Most suckers observed on 20 week-old plants were peepers (i.e. small sucker with scale leaves) or sword suckers (i.e. larger sucker with lanceolate leaves). These suckers had their own root system, confirming observations by Robin and Champion (1962) and Beugnon and Champion (1966) who reported that sword suckers of the dessert banana Poyo had a well-developed root system. The observed positive effect of ploidy on cord root diameter confirms earlier observations by Monnet and Charpentier (1965).

Shoot to root ratios depend on the developmental stage of a plant (Brouwer 1966). In the case of banana, Gousse- land (1983) estimated banana cord root number from leaf area and reported an effect of plant developmental phase on the accuracy of the regression model. The author reported an underestimation of the number of cord roots during the early vegetative phase. Hence, regression models will have to be fine-tuned according to the developmental phase of a plant.

In addition, shoot-root ratios are highly influenced by environmental conditions (Brouwer and De Wit 1969, Squire 1993, Martínez García 1997, McMichael and Burke 1998). Therefore, fine-tuning of these models is needed when growing plants under different environmental conditions.

This study shows that *Musa* root system development can be estimated from shoot growth characteristics. This provides a means of relating above-ground development to root growth, which may prove useful for non-destructive assessment of root development.

Acknowledgements

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References


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of 1000 bunches per hectare, representing a commercial value of close to 3 million pesos (US$1500). The disease could reduce production by nearly 70% and cause serious problems in zones with a rural economy (Echeverry, unpublished data).

The symptoms of the disease consist of chlorosis in the lower leaves followed by wilting at the petiole and then ascending wilt that finally affects all the leaves of the plant (Figure 1). A cross-section of an infected pseudostem about 1 m above ground level reveals watery rot and an unpleasant smell (Figure 2). In addition, the colour of the inner leaf sheaths ranges from brown to dark brown (Figure 3).

Unlike the symptoms of the pseudostem soft rot observed by Guzmán and Sandoval (1996) in FHIA-01 and FHIA-02 hybrids, the infection described at Icononzo moves from the rhizome towards the upper part of the plant.

In a study on pseudostem soft rot in FHIA hybrids with similar symptoms to those observed in Dominico hártón plantain, Guzmán and Sandoval (1996) isolated the Erwinia carotovora bacterium from plant tissues. The soft or watery rots caused by Erwinia spp. bacteria are often observed in Musaceae in Latin America (Stover 1972).

Stover (1972) noted that the bacteria E. chrysanthemi and E. carotovora affect the corm and pseudostem of both banana and plantain and observed that cultivars of the Cavendish subgroup are susceptible to Erwinia sp. but that the AAB and ABB genotypes are more tolerant.

Rivera and Ezavin (1989) observed pathology characterized by corm disease in Musa acuminate cultivars (AAA) in different banana zones in Venezuela. The pathogen was found to be the bacterium E. chrysanthemi Burk. et al.

Cedeño et al. (1990) reported that the bacterium E. carotovora subsp. atroseptica is the pathogen of soft pseudostem rot in 'Hartón' plantain (Musa AAB) in the southern Maracaibo lake area.

In his list of the main diseases of Musaceae crops in Zulia State in Venezuela, Urdaneta (1994) mentions E. carotovora as the causal agent of plantain pseudostem rot.

Jiménez et al. (1994) reported that E. chrysanthemi Burk. et al. is the causal agent of plantain corm necrosis. The same authors isolated three strains of bacteria in the rhizosphere of apparently healthy plantains from a field in which the symptoms of corm necrosis were not strongly developed. These bacteria were antagonistic in vitro to isolates of E. chrysanthemi and E. carotovora. The morphological, physiological and biochemical characteristics of the strains showed that they belonged to the genus Pseudomonas.

Belalcazar et al. (1991) pointed out that the bacterium E. chrysanthemi p.v. paradisiaca Victoria and Barros is endemic in the regions in which Musaceae are grown and that attacks are enhanced by drought and nutritional deficiencies in plantations.

Schneider’s observations (1991) of the relation between mineral nutrition and host condition showed that all K, Ca and Mg applications limited the development of certain types of wilt, and especially those caused by Fusarium sp. In the absence of KCl, the exudate produced causes greater reduction of sugars and organic acids. KCl reduces the infection level. Mineral nutrition therefore has a distinct effect on the nature of the exudate and on infection. Trichoderma can inhibit the pathogen via its antibiotic substances or by breaking down bacterial cell walls by means of enzymes such as quinonases, B-1,3-glucanases, proteases, mannases and hydrolases (Limón et al. 1999).

The relative importance of these two mechanisms in the antagonist process depends specifically on host-pathogen interactions (Limón et al. 1999). Nevertheless, the combination of the action of hydrolytic enzymes and antibiotic substances in Trichoderma has demonstrated its antifungal synergy (Schirmbock et al. 1994).

Materials and methods

The study was performed from 1997 to 1999 in Icononzo in the Piedecuesta region in Tolima Department in Colombia. The experiment was conducted at San Isidro at an elevation of 1380 m, with annual average precipitation of 1500 to 1700 mm and 80% average relative humidity. The soil is slightly acid clay loam with a moderate percentage of organic matter and little potassium.

Samples for laboratory analysis and identification of the pathogen were taken from the inner part of pseudostem of Dominico hártón plantain (Musa cv. AAB) with symptoms of infection. The samples were first washed in running water and then cut into small 2 cm lengths and then disinfected with sodium hypochlorite 2.5% for 3 minutes under constant agitation. They were washed in sterile distilled water to remove residues of hypochlorite.

Once disinfected, the samples are macerated in a mortar containing 1 ml
Table 1. Summary of the results of the morphological and physicochemical tests performed to characterize the bacterium isolated from pseudostems of 'Dominico hartón' plantain (AAB).

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram</td>
<td>-</td>
</tr>
<tr>
<td>KOH 3% to confirm Gram test</td>
<td>+</td>
</tr>
<tr>
<td>odour</td>
<td>foetid</td>
</tr>
<tr>
<td>colour</td>
<td>cream</td>
</tr>
<tr>
<td>consistency</td>
<td>butyry</td>
</tr>
<tr>
<td>Congo red</td>
<td>rod-shaped</td>
</tr>
<tr>
<td>fluorescence on King-B</td>
<td>-</td>
</tr>
<tr>
<td>catalase</td>
<td>+</td>
</tr>
<tr>
<td>levan</td>
<td>-</td>
</tr>
<tr>
<td>O-F test (Hugh-Leifson)</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of gelatine</td>
<td>+</td>
</tr>
<tr>
<td>NaCl 3%</td>
<td>+</td>
</tr>
<tr>
<td>NaCl 4%</td>
<td>+</td>
</tr>
<tr>
<td>tetracycline</td>
<td>+</td>
</tr>
<tr>
<td>streptomycin</td>
<td>+</td>
</tr>
<tr>
<td>penicillin</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>genus</th>
<th>Erwinia</th>
</tr>
</thead>
</table>

+ = positive; - = negative.

sterile distilled water. LB (Luria-Bertani) medium (yeast extract 5 g/l, tryptophan 10 g/l, NaCl 10 g/l, agar 20 g/l, pH 5.5-6.0) was added to 50 µl of this macerated preparation. The Petri dishes were then placed in an incubator (Precision Scientific Inc.) and kept at 28°C for 48 hours. The following tests were performed to characterize the pathogen: Gram stain, confirmation of Gram by KOH 3%, catalase, hydrolysis of gelatine, levan, King-B, OF test (Hugh-Leifson), tolerance to NaCl 3% and 4%, antibiogram with tetracycline, streptomycin and penicillin.

Three plots were marked out in a 2600-m² commercial holding planted with 29-month Dominico hartón plantain displaying symptoms of vascular wilt; three planting layouts were used: 5, 4 and 3 metres between rows and 2.5 metres between plants. Four subplots of 5 plants each with three repetitions were laid out for each planting distance.

Four treatments were applied to each subplot.

The experiment was conducted in two phases:

- **Phase I**, from November 1997 to August 1998 (8 months), during which the following treatments were applied and evaluated:
  - chemical treatment (T1). Monthly spraying of Vanodine 5 cc per 1 water (composition: c/100 ml: surfactant iodine compound; 2.5% available iodine. Pfizer brand) around the pseudostem of each plant;
  - cultural treatment (T2). Application of the following fertilizers at the specified doses: urea (46%) 150 g/plant, KCl (60% K, O) 200 g/plant, DAP (di ammonium phosphate: P₂O₅ 48%, N'18%) 66 g/plant and "Micronfós" (Microfertica,Colombia)200 g/plant; dolomitic limestone was applied at 300 g/plant to correct the pH;
  - biological treatment (T3). Kasumin 2% [(Kasugamycin: 3-O-(2-amino-4-(1-carboxyformidylo) amino 2, 3, 4, 6 tetra oxy-alpha-D-arabino hexapyranosyl)inositol] at 1 cc per L water was applied around the pseudostem;
  - control (T4). No substances were applied.

- **Phase II**, from September 1998 to February 1999 (6 months). Observation of the results was followed by modification of the three treatments applied in Phase I. The following treatments were applied and evaluated in Phase II:
  - chemical treatment (T1). Injection using a plastic syringe with a hypodermic needle of 5 cc Vanodine in four positions in the pseudostem of each plant at 1 m above ground level;
  - cultural treatment (T2). Solely the application of potassium in KCl form at 200 g/plant every 30 days. Application was performed at three points around the plant 50 cm from the base of the pseudostem;
  - biological treatment (T3). At the beginning of the second vegetative cycle, a single inoculation of the fungus Trichoderma spp., with the strain being isolated from soils infected plants and multiplied in the laboratory on the selective medium proposed by Elad et al. (1987). Doses of 50 g/plant were placed in the soil at four points around the pseudostem;
  - control (T4). No substances were applied.

Results and discussion

Laboratory results

Morphological and physicochemical tests were performed to determine the causal agent of vascular rot. The morphological Gram test revealed pink bacilli characteristic of Gram-negative bacteria.

Congo red staining was also performed to observe the rod-shaped bacterial cells.

To confirm the Gram stain test, a fragment of bacterial growth was placed on a slide and a drop of KOH 3% was added. The formation of a viscous, mucilaginous suspension was observed. This positive reaction confirmed that the bacterium is Gram-negative.

Among the physicochemical tests performed, growth on King-B medium to characterize fluorescent bacteria gave a negative result. The catalase test consisting of emulsifying a portion of bacterial growth with hydrogen peroxide (H₂O₂) 10% gave a positive result. The gelatine test was positive, indicating the presence of proteolytic enzymes in the bacterium. The oxidation-fermentation test (OF) on Hugh and Leifson medium was positive and detected production of acids by oxidation in aerobicosis. The growth test on nutrient gelose with NaCl 3% and 4% was positive, with the colonies growing normally.

Reaction to the antibiotics tetracycline, streptomycin and penicillin was tested using antibiotic assay medium No. 5 , pH 8.0 ± 0.1 (meat extract 1.5 g/l, yeast extract 3.5 g/l, meat peptone 6.0 g/l, agar-agar 15.0 g/l). Bacterial growth in saline solution (1 ml) was spread on the medium with a "rake" type glass spreader before the discs of antibiotics were placed. The reaction was positive for tetracycline and streptomycin and negative for penicillin. This means that the bacterium was very sensitive to the first two antibiotics, as shown by the transparent halo free of bacterial growth around the two discs concerned.

Analysis of the results of these tests performed on bacterial samples from the pseudostems of plantains infected by bacterial rot showed that the colonies consisted of Erwinia sp. (Table 1).

INFOMUSA — Vol 10, N°1 19
Field results
It is important to note that there are no significant differences between average numbers of healthy leaves and average numbers of wilted leaves in the main Phase I plots. This means that plantation densities are not a factor in enhancing the upward wilting of leaves on the plants (Table 2). The same trend with regard to average healthy leaves was observed in the main plots during Phase II. In contrast, significant differences were observed in the average numbers of wilted leaves, as shown in Table 2.

Although Phases I and II are statistically similar, there is a marked difference in the average numbers of healthy and wilted leaves per plant. Whereas an average of 2.84 healthy leaves was observed in Phase I, the figure attained 7.77 in Phase II. The same applies to wilted leaves with 0.76 in Phase I and 1.40 in Phase II.

This may be explained by the change in fertilization from urea + DAP + “Micronfos” to KCl alone. On this subject, according to Jacob et al. (1961), the nutrients taken up by plantain include very large amounts of potassium.

The same author observed that since it is an extremely potassium-hungry plant, certain considerations concerning the application of other nutrients such as Ca and Mg must be taken seriously. Indeed, it has been demonstrated that excess potassium causes the physiological disorder referred to as “blue disease” and yellow pulp, affecting fruit quality.

It is also important to note that the average number of wilted leaves was lower in the control than in the other treatments. This may be explained by the fact that the control plants did not have a normal number of leaves, that leaf emission was small and that practically all the leaves were infected (Table 3). The same phenomenon was observed in the response to Phase II treatments. The cultural control treatment was better than the biological and control treatments. However, it should be stressed that there is a difference of 4.92 in the average number of healthy leaves in the two phases, that is to say that there were more healthy leaves in Phase II than in Phase I (Table 3).

Various hypotheses might account for these differences in behaviour. The most acceptable concerns the synergy between the presence of the fungus Trichoderma sp. and the ability of the plant to absorb nutrients from the soil.

This was verified by observation of the leaf size and colour in ‘Dominico hartón’ plantain; leaf size and the intensity of green were greater in Phase II leaves than in Phase I leaves. The fungus Trichoderma sp. has a positive effect on weight, size and leaf and flower production (Chet 1987).

On this subject, Kleifel et al., quoted by Chet (1987), observed earlier germination and increased leaf length, width and dry weight in melon, tomato, cucumber, radishes and bean plants.

It was observed that the cultural treatment gave the best results in both Phase I and Phase II for the number and average weight of commercial bunches harvested (Table 4).

There were no significant differences in commercial bunch weights in the main plots during each phase of the experiment but the differences between subplots were very highly significant. This demonstrates the effectiveness of the treatments, and especially the cultural treatment in the provision of nutrients via fertilization.

Furthermore, no significant differences were found in the interaction between planting differences and treatments (PP*SP), as is shown by the results of analysis of variance in Table 5.

The analysis shows that the cultural treatment displays significant differences at 5% in comparison with the chemical and biological treatments and the control for the “harvested bunch weight” variable.

This confirms Machado’s observation quoted by Jacob et al. (1961) that the greater part of potassium absorption (84%) takes place during the fruit formation period. The same author calculated that a plantation of 1333 plants per hectare absorbed approximately 3493 kg potassium in 14 months.

There were no significant differences between the chemical and biological treatments for the same “harvested bunch weight” variable but there were differences with respect to the control.

The chemical and biological treatments displayed very similar “harvested bunch weight” results in Phases I and II of the experiment. However, several deviations might result from the number of functional leaves at flower emission, a determinant parameter for fruit weight and quality (Belalcázar et al. 1991).

Conclusions
The causal agent of rot and vascular wilt of plantain is the bacterium Erwinia, and probably the species E. carotovora.

Planting densities have no effect on the presence or not of vascular rot in the plantain crops.

Potassium-rich fertilization and the presence of the fungus Trichoderma sp.
help to maintain plant vigour and reduce the probability of attack by pathogenic microorganisms.

Although chemical (Vanodine) and biological (Kasugamycin 2%) treatments do not contribute directly to the control of plantain vascular rot, they contribute to preventing the spread of the problem.

The frequent use of sodium hypochlorite in commercial form (50% dilution in water) is an essential practice for disinfecting tools and should be strongly encouraged.

Acknowledgements
The authors thank Mr Alfonso Guerrero Gacha, the owner of San Isidro farm at Icononzo, for all the facilities that he placed at their disposal during the field experiment. They also thank Mr Antonio Maria Caicedo, engineer at the CORPOICA Nataima center, for his collaboration in the statistical analysis of the results.

References


Evaluation of FHIA hybrids in comparison with local Musa clones in a black Sigatoka-free area of eastern Peru

U. Krauss, W. Soberanis and J. Jarra

The International Musa Testing Programme (IMTP) aims at comparing improved Musa germplasm, most notably FHIA hybrids, with popular clones in over 50 countries worldwide (Ojeda et al. 1999). Peru has not participated in this effort. The limited information available on Musa production in Peru was reviewed by Krauss et al. (1999) and the authors recommended germplasm trials with FHIA hybrids and popular and/or high-yielding local clones.

Diseases aggravated by almost complete lack of control measures are the most limiting factor for Musa production in Peru. Black Sigatoka is present in part of the production area only (Krauss et al. 1999). Elsewhere, yellow Sigatoka and Cordana leafspot are the most important diseases. Neither has received major attention from breeders because black Sigatoka is of greater importance on an international scale. In fact, few recent evaluations on yellow Sigatoka resistance are...
<table>
<thead>
<tr>
<th>Hybrid or Clone</th>
<th>Genomic make-up</th>
<th>Subgroup (int. clone)</th>
<th>Reported disease reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA-01</td>
<td>AAAB</td>
<td>Hybrid</td>
<td>Resistant</td>
</tr>
<tr>
<td>FHIA-03</td>
<td>AAB</td>
<td>Hybrid</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ingui</td>
<td>AAB</td>
<td>French Plantain</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Bellaco</td>
<td>AAB</td>
<td>Horn Plantain (Harton)</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Isla del Alto Huallaga</td>
<td>ABB</td>
<td>Pisang Awak?</td>
<td>Moderately resistant</td>
</tr>
</tbody>
</table>

1 Krauss et al. (1999) reported a wide range of disease reactions by local clones to black and yellow Sigatoka. The reaction listed here represents the conclusion reached in their article.
2 nd, no data.

Table 1. Musa hybrids and clones included for this study in a black Sigatoka-free area of eastern Peru. Disease reactions were summarized from Krauss et al. (1999) for Peru and, when not available, from Jones (2000).

Results

Table 3 shows the agronomic characteristics of the Musa clones and hybrids in this study. Isla had the shortest production cycle with significantly fewer days from planting to flowering and harvest than the other varieties. In the raton crop, this phenomenon was even more pronounced. FHIA-A01 had the second fastest harvest-to-harvest cycle, FHIA-A03 and Ingui were intermediate, and Bellaco had the longest harvest-to-harvest interval. Isla also had the highest leaf production rate in the plant crop although this failed to reach statistical significance. Therefore, this parameter was not analyzed for the raton crop.

All varieties were marginally taller at harvest than at flowering; all except FHIA-A01 in the plant crop also showed a slight increase of pseudostem perimeters from flowering to harvest. Bellaco was the tallest clone, followed by Ingui. These clones also had the thickest pseudostems. The two FHIA hybrids were in the same height range at flowering and harvest. Isla was the shortest clone with the thinnest pseudostem. Pseudostem circumference increased in the order Isla, FHIA-A01, FHIA-A03, Ingui, Bellaco. This trend became more pronounced with time (Table 3).

The number of functional leaves was very similar to the total number of leaves. In the plant crop, varieties retained between 93% (Ingui) and 100% (FHIA-03) of leaves at flowering. All retained over 98% at harvest (Table 3). This apparent “increase” in plant health over time is due to the fact that defoliation was practised during the later stages of plant development only (Table 2) so that non-functional leaves were removed more diligently closer to harvest. The more realistic indicator of disease progress is the decrease of both total and functional leaves from flowering to harvest. In the plant crop, this was most pronounced for Ingui which lost 23.6% of total leaves and 18.1% of functional leaves between flowering and harvest. Bellaco lost

Materials and methods

The reported disease reactions of the germplasm used in this study are shown in Table 1. The trial sites and their characteristics are given in Table 2. Fields were located in the “cocoa-belt” of the upper Huallaga valley. All cooperating farmers actively expressed interest in participating in “alternative crops” activities and had years of experience in Musa production in adjacent fields. Trials were designed in a participatory manner: no attempt was made to optimize or standardize agronomic practices; these were left at the discretion of the farmer (Table 2). We believe this to be the most informative approach to germplasm evaluation under local conditions because it encompasses local crop husbandry practices. It also increases data variability; therefore, ten plants per plot were used rather than the recommended four to six. In other aspects, the INIAB guidelines for germplasm evaluation (Orjeda 1998) were followed. Tissue-cultured FHIA hybrids were the courtesy of FHIA. After hardening in a greenhouse, the plantlets were transplanted at a spacing of 3 m x 3 m as practised for local clones. Trials followed the randomized block design with one incomplete block: Bellaco was not planted in the field in Marona.

Shortly after installation, the plot in Cotonomillo was abandoned because of terrorist activity and no data are available. Evaluation elsewhere took place in fortnightly intervals. For the plant crop, key parameters were recorded six months (182 days) after planting, at flowering and at harvest. The maximum leaf production rate was calculated from Gompertz-curves constructed by logistic regression on Genstat 5. For the raton crop, key parameters were recorded at flowering and harvest only. The second raton of Isla was compared with the first raton of the other cultivars because these coincided in terms of timing and, thus, the concomitant seasonal fluctuations of climatic conditions, disease pressure and marketing potential, whereas the first raton crop of Isla was already harvested before any of the other varieties flowered.

available because black Sigatoka replaces yellow Sigatoka except at high altitudes and existing data are highly variable. Resistance to yellow Sigatoka is not correlated with black Sigatoka resistance (Jones 2000). We found no comparative study on Cordana leafspot.

Yellow Sigatoka is caused by Mycosphaerella musicola Leach and is distributed almost worldwide. In the absence of black Sigatoka caused by Mycosphaerella fijiensis Morelet, yellow Sigatoka can cause major losses, especially on AAA bananas. Plantains (AAB) are resistant to yellow Sigatoka at sea level. At higher altitudes, however, especially under poor growing conditions, they are susceptible. ABB clones, including Pisang Awak, are regarded to be resistant (Jones 2000). Both the AAB and ABB groups are affected by yellow Sigatoka in Peru under poor management (Krauss et al. 1999).

Cordana leafspot is caused by Cordana musae (Zimmermann) Höhnel and is distributed worldwide. Although it is of minor importance internationally, it can cause severe defoliation especially on plantain. Wet weather and debilitated plants favour the disease. Both factors are prevalent in Peru, where up to 4000 mm of rainfall in some growing areas and the lack of drainage and sucker control as well as pests weaken the plants. C. musae attacks several Musa spp. and Ensete glaucum; most Musa subgroups are regarded to be susceptible (Jones 2000).

The objective of this study was to compare the improved hybrids FHIA-01 (AAAB) and FHIA-03 (AABB) with the local clones Ingui (AAB, French plantain), Bellaco (AAB, Horn plantain “Harton”) and Isla del Alto Huallaga (ABB, Pisang Awak subgroup) in agronomic, pathological and economical terms.
Leaf loss was directly related to disease susceptibility (Table 4). FHIA hybrids, especially FHIA-03, were least susceptible to both yellow Sigatoka and Cordana leafspot whether measured as average severity or infection indices at different times. In the plant crop, Inguri and

Table 2. Description of the farmer-managed germplasm trial plots.

<table>
<thead>
<tr>
<th>Location</th>
<th>Planting date</th>
<th>Farmers</th>
<th>Researchers</th>
<th>Agronomic practices (days after planting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotomominio</td>
<td>19/03/98</td>
<td>Good Musa soil;</td>
<td>Fertile, alluvial soil, flood-prone; 30 years under banana/plantain without</td>
<td>Weed control and deleafing (181, 234, 503)</td>
</tr>
<tr>
<td>(Aucayacu)</td>
<td></td>
<td>Wind damage</td>
<td>fertilization, but with plant materials as mulch.</td>
<td>Deleafing (292, 345, 651)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stem borer damage; Furadan used occasionally.</td>
<td>Borer control (Furadan) (234)</td>
</tr>
<tr>
<td>Pendencia</td>
<td>19/03/98</td>
<td>Good Musa soil;</td>
<td>Fairly fertile soil, flood-prone, 3rd year under Musa; plant materials used</td>
<td>Weed control, deleafing (260, 281, 425)</td>
</tr>
<tr>
<td>(Fondo Bazán)</td>
<td></td>
<td>Wind damage</td>
<td>as mulch; previously cocoa. Stem borer damage; Furadan used occasionally.</td>
<td>Deleafing (325, 437, 589)</td>
</tr>
<tr>
<td>Marona</td>
<td>23/3/98</td>
<td>Fair Musa soil;</td>
<td>Fertile, alluvial soils; no risk of inundation.</td>
<td>Weed control and deleafing (193, 263, 316)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stem borer</td>
<td>Stem borer damage; Fusarium sp. has yet to be confirmed in this field.</td>
<td>542, 625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;Seca seca&quot; (Fusarium</td>
<td>Application of lime in 1997 and Furadan in 1998.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wilt)</td>
<td>First year under banana following papaya.</td>
<td></td>
</tr>
<tr>
<td>Pendencia</td>
<td>16/4/98</td>
<td>Good Musa soil;</td>
<td>Fertile alluvial soils, lack of natural drainage,</td>
<td></td>
</tr>
<tr>
<td>(Fondo Magnos)</td>
<td></td>
<td>Stem borer</td>
<td>flood-prone. Stem borer damage confirmed.</td>
<td>Weed control (22, 83, 338)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>First year under Musa.</td>
<td>Deleafing (464)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Weed control and deleafing (193, 263, 316)</td>
</tr>
</tbody>
</table>

Table 3. Agronomic characteristics of FHIA hybrids as compared with those of Peruvian clones

<table>
<thead>
<tr>
<th>Plant crop</th>
<th>FHIA-01 (AABB Hybrid)</th>
<th>FHIA-03 (AABB Hybrid)</th>
<th>Inguiri (French Plantain)</th>
<th>Bellaco (Hom Plantain)</th>
<th>Isla1 (Pisang Awak?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to flowering</td>
<td>268 b</td>
<td>279 b</td>
<td>279 b</td>
<td>284 b</td>
<td>198 b</td>
</tr>
<tr>
<td>Days to harvest</td>
<td>390 b</td>
<td>411 b</td>
<td>403 b</td>
<td>410 b</td>
<td>299 a</td>
</tr>
<tr>
<td>Height at flowering (cm)</td>
<td>239 b</td>
<td>233 b</td>
<td>268 c</td>
<td>311 d</td>
<td>201 a</td>
</tr>
<tr>
<td>Height at harvest (cm)</td>
<td>243 b</td>
<td>238 b</td>
<td>287 c</td>
<td>320 d</td>
<td>203 a</td>
</tr>
<tr>
<td>Circumference of pseudostem at flowering (cm)</td>
<td>77.3 h</td>
<td>88.7 b</td>
<td>91.7 b</td>
<td>109.8 c</td>
<td>59.4 b</td>
</tr>
<tr>
<td>Circumference of pseudostem at harvest (cm)</td>
<td>76.5 h</td>
<td>93.7 c</td>
<td>101.2 c</td>
<td>114.1 d</td>
<td>62.5 a</td>
</tr>
<tr>
<td>Total number of leaves at flowering</td>
<td>10.8 h</td>
<td>11.1 b</td>
<td>8.9 a</td>
<td>8.1 a</td>
<td>7.9 a</td>
</tr>
<tr>
<td>Total number of leaves at harvest</td>
<td>9.9 h</td>
<td>10.3 b</td>
<td>6.8 a</td>
<td>6.7 a</td>
<td>6.7 a</td>
</tr>
<tr>
<td>Loss of total leaves from flowering to harvest (%)</td>
<td>8.3</td>
<td>7.2</td>
<td>23.6</td>
<td>17.3</td>
<td>15.2</td>
</tr>
<tr>
<td>Functional number of leaves at flowering</td>
<td>10.7 b</td>
<td>11.1 b</td>
<td>8.3 a</td>
<td>8.0 a</td>
<td>7.7 a</td>
</tr>
<tr>
<td>Functional number of leaves at harvest</td>
<td>9.9 b</td>
<td>10.1 b</td>
<td>6.8 a</td>
<td>6.7 a</td>
<td>6.6 a</td>
</tr>
<tr>
<td>Loss of functional leaves from flowering to harvest (%)</td>
<td>7.5</td>
<td>9.0</td>
<td>18.1</td>
<td>16.2</td>
<td>14.3</td>
</tr>
<tr>
<td>Maximum leaf production rate (leaves per week)</td>
<td>0.28 a</td>
<td>0.42 a</td>
<td>0.41 a</td>
<td>0.35 a</td>
<td>0.64 a</td>
</tr>
</tbody>
</table>

Ratoon crop

| Days from flowering to flowering | 278 b | 286 b | 283 b | 296 b | 150 a |
| Days from harvest to flowering | 156 b | 150 c | 154 b | 164 b | 49 a |
| Days from harvest to harvest | 265 b | 276 b | 276 b | 296 b | 149 b |
| Height at flowering (cm) | 245 b | 244 b | 291 b | 328 b | 211 b |
| Height at harvest (cm) | 250 b | 248 b | 296 c | 334 d | 216 b |
| Circumference of pseudostem at flowering (cm) | 72.9 | 86.7 b | 98.7 c | 115.1 d | 62.2 a |
| Circumference of pseudostem at harvest (cm) | 77.2 b | 90.0 c | 102.0 d | 115.5 e | 65.5 b |
| Total number of leaves at flowering | 10.2 h | 10.4 b | 7.7 a | 8.5 a | 7.9 a |
| Total number of leaves at harvest | 9.7 h | 9.8 b | 7.2 a | 7.5 a | 7.2 a |
| Loss of total leaves from flowering to harvest (%) | 4.9 | 5.8 | 6.5 | 11.8 | 8.9 |
| Functional number of leaves at flowering | 10.0 b | 10.3 b | 7.7 a | 8.5 a | 7.7 b |
| Functional number of leaves at harvest | >9.5 b | 9.7 b | 7.2 a | 7.4 a | 7.0 a |
| Loss of functional leaves from flowering to harvest (%) | 5.0 | 5.8 | 6.5 | 12.9 | 9.1 |

1 The second ratoon of Isla was compared with the first ratoon of the other clones and hybrids.

**Values within a row followed by the same letter do not differ at P < 0.05 (Tukey test).
### Table 4. Leafspot reaction of FHIA hybrids as compared with those of Peruvian clones.

<table>
<thead>
<tr>
<th>Plant crop</th>
<th>FHIA-01 (AAAB Hybrid)</th>
<th>FHIA-03 (AAAB Hybrid)</th>
<th>Inguiri (French Plantain)</th>
<th>Bellaco (Horn Plantain)</th>
<th>Isla$^3$ (Pisang Awak?)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yellow Sigatoka</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average severity (%)</td>
<td>0.54 $^a$</td>
<td>0.12 $^a$</td>
<td>4.09 $^c$</td>
<td>0.69 ab</td>
<td>2.57 bc</td>
</tr>
<tr>
<td>Infection Index 6 months after planting</td>
<td>0.53 $^a$</td>
<td>0.00 $^a$</td>
<td>4.37 $^b$</td>
<td>0.58 a</td>
<td>4.36 b</td>
</tr>
<tr>
<td>Infection Index at flowering</td>
<td>1.09 $^a$</td>
<td>0.56 $^a$</td>
<td>3.55 $^b$</td>
<td>0.82 a</td>
<td>3.73 b</td>
</tr>
<tr>
<td>Infection Index at harvest</td>
<td>1.67 $^a$ $^{ab}$</td>
<td>0.25 $^a$</td>
<td>7.45 $^c$</td>
<td>3.11 b</td>
<td>6.73 c</td>
</tr>
<tr>
<td><strong>Cordana leafspot</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average severity (%)</td>
<td>1.21 $^a$</td>
<td>0.95 $^a$</td>
<td>2.00 $^{ab}$</td>
<td>2.96 b</td>
<td>1.57 ab</td>
</tr>
<tr>
<td>Infection Index 6 months after planting</td>
<td>1.73 $^a$</td>
<td>1.26 $^a$</td>
<td>2.37 $^{ab}$</td>
<td>3.62 b</td>
<td>2.52 ab</td>
</tr>
<tr>
<td>Infection Index at flowering</td>
<td>1.85 $^a$ $^{ab}$</td>
<td>0.92 $^a$</td>
<td>3.01 $^b$</td>
<td>2.67 b</td>
<td>3.00 b</td>
</tr>
<tr>
<td>Infection Index at harvest</td>
<td>2.28 $^a$ $^{ab}$</td>
<td>1.95 $^a$</td>
<td>3.55 $^b$</td>
<td>5.88 c</td>
<td>5.04 bc</td>
</tr>
</tbody>
</table>

---

Isla were most affected by yellow Sigatoka. Bellaco was most susceptible to Cordana leafspot, closely followed by Inguiri and Isla. In the ratoon crop, all cultivars were affected more by yellow Sigatoka than by Cordana leafspot but disease severity and indices were more variable. Inguiri, followed by Isla, exhibited the highest yellow Sigatoka severity. Other parameters and Cordana leafspot did not reach statistical significance (Table 4). In contrast to the plant crop in which disease increased over time, in the ratoon crop, yellow Sigatoka in Inguiri and, even more pronounced, both diseases in Bellaco seem to decrease from flowering to harvest. This can be attributed to deleping just before these clones were harvested (Table 2) and is also in agreement with the high percentage leaf loss of Bellaco from flowering to harvest (Table 3).

An economic consideration (Table 5) indicates that FHIA-03 was the overall most lucrative Musa variety during the first production cycle after planting. It produced the most fingers per bunch and attracted a high price, which is calculated on a per 1000 finger basis in eastern Peru. Large bunches combined with a fast production cycle made Isla the second most economical clone. In the ratoon crop, Isla surpassed FHIA-03 in terms of economics due to a remarkably fast ratooning and flowering rate (Table 3). These characteristics compensated for its susceptibility to yellow Sigatoka and a somewhat lower price. FHIA-01 was consistently the third most economical hybrid because of its large bunches and disease resistance. It fell into the same price-class as Isla. Inguiri was less profitable. Bellaco was least economical. The lower economic returns for these two plantain clones were because of their small bunches (Table 5) and high disease susceptibility (Table 4) which outweighed a high per-finger price. All varieties became more profitable in the ratoon crop, although farm-gate prices for all varieties, except Bellaco, had dropped.

### Discussion

We deliberately chose a participatory approach to germplasm evaluation because we believe this to be the most realistic representation of local growing conditions. Both agronomic practices (or the lack of them) and personal preferences for a variety could be included in the evaluation. Interestingly, one farmer decided not to plant Bellaco. This clone later proved to be the worst in the overall performance. Nevertheless, the collaborating farmers were the more conscientious ones of that area with interest and experience in Musa production. We are aware that crop management was above the regional average.

None of the participating farmers recognized fungal diseases as limiting factors for production. Instead, they were regarded as normal leaf appearance. All growers had problems with toppling and attributed this to wind, which is negligible in the area, or weevil attack, which is ubiquitous. Average farmers do not usually recognize this pest. The complete lack of drains in flood-prone fields further contributed to weakened root systems. Nematodes were no major problem as all fields except the lost plot in Cotonomillo were only recently planted to Musa (Table 2). One farmer used insecticide during the duration of the trial. All practised manual weed control and deleping. The latter only commenced about six months after planting when flowering of Isla had begun and resumed before the harvest peak of both production cycles (Tables 2 and 3). Little attention was paid to the crop during early development stages, i.e. when the bunches differentiated and the number of fingers,
on which the pricing structure is based, were determined.

FHIA-01 was bred as a dessert banana substitute. It is the only hybrid with simultaneous resistance to black Sigatoka, Panama disease and crown rot. Furthermore, it is high-yielding even under poor conditions including drought (FHIA 2000). FHIA-01 tends to perform better in subtropical than tropical conditions, especially with respect to fruit quality (Jones 2000). FHIA-03 was bred as Bluggoe substitute. It is resistant to black Sigatoka and Moko disease. It is a sturdy hybrid which yields well under adverse conditions such as poor soils and drought. Its main weakness is the short greenlife. FHIA-03 is therefore recommended for homegardens and local consumption (FHIA 2000). Boiling time for cooking green FHIA-03 is only half that required for Bluggoe (Jones 2000).

In the black Sigatoka-free area where this study was conducted, FHIA-03 performed similar to the best local clone (Isla) and FHIA-01 also compared very well. The Huallaga valley is a highly tropical environment and poor drainage rather than drought is a problem (Krauss et al. 1999). Under these circumstances, it is gratifying to see FHIA hybrids perform so well and be accepted by local and metropolitan markets at the same time. Especially, the success of FHIA-03 is surprising. Bluggoe is not appreciated in Peru (Krauss et al. 1999), but farmers reported multipurpose uses for FHIA-03.

The study also indicated that Isla del Alto Huallaga is moderately susceptible to yellow Sigatoka in eastern Peru. This finding contradicts Table 1, but may help to resolve a point of contention: Isla has been classified as susceptible to highly resistant and Inguiri and Bellaco as susceptible to resistant by different authors. Krauss et al. (1999) concluded that Isla is resistant and Inguiri and Bellaco are moderately resistant to yellow Sigatoka but these resistance reactions are dependent on the growing conditions. All three clones are reported as susceptible to Cordana leafspot (Krauss et al. 1999). According to the plant crop data, Isla and Inguiri are similarly susceptible to yellow Sigatoka, whereas Bellaco may be less susceptible to yellow Sigatoka but more so to Cordana leafspot. However, in the ratoon crop, all varieties suffered more from yellow Sigatoka than from Cordana leafspot. The high variability of disease incidence in the ratoon crop, especially near harvesting, may be due to increased phytosanitary pruning around that time.

Isla has recently been incorporated into FHIA's breeding programme (Phil Rowe, pers. comm., 2000). It would be worthwhile to investigate whether aggressive pathotypes of M. muscosa have evolved in the Huallaga valley and/or to corroborate the affinity of Isla clones with Pisang Awak. Isla has also been suggested to belong to the Ichola (AAB) subgroup (Thierry Lescot, pers. comm., 1999). Furthermore, "Isla" is the collective term for five to seven distinct clones within one group (Krauss et al. 1999). It is conceivable that their disease reactions differ.

The present trials show that the FHIA germplasm exhibits resistance to yellow Sigatoka and Cordana leafspot under mediocre growing conditions and management in eastern Peru. FHIA-03 was least susceptible to diseases, produced the largest bunches, and was rated in the highest price class. This suggests that this clone has excellent marketing potential for Peruvian internal markets. Bellaco commanded a similar price to FHIA-03 (for 1000 fingers), but Bellaco was the least economical because of its small bunch sizes. FHIA-01 was less popular but also has good potential. It fell into the same price class as Isla, one of the most popular clones in Peru (Krauss et al. 1999). Only the most favoured local clones were included in this study, and it is a great achievement for a new variety to compete with any of these on the market during the first year of planting. Participating farmers also reported good markets for FHIA planting material. However, none of them was prepared to sell followers of FHIA-03 which produces fewer suckers than FHIA-1. Instead, farmers expanded their own area under FHIA-3.

We can wholeheartedly recommend the introduction of FHIA hybrids on a larger scale in Peru, especially in view of the constant expansion of black Sigatoka. Evaluations of FHIA hybrids in areas affected by black Sigatoka are ongoing (Phil Rowe and Raul Anguiz, pers. comm., 1999).

Acknowledgements

This paper was prepared during a diversification project funded by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and managed by CABI Bioscience. During the first three months, additional funds were received from the Organization of American States (Inter-American Drug Abuse Control Commission, IADCC/OAS). FHIA germplasm was generously provided by Phil Rowe. The authors also wish to thank colleagues at CABI (Commonwealth Agricultural Bureau International, UK), CIRAD-FLHOR (Centre de coopération internationale en recherche agronomique pour le développement-Département des productions fruitières et horticole, France), FAO (Food and Agricul-

<table>
<thead>
<tr>
<th>Table 5. Economic returns of FHIA hybrids as compared with those of Peruvian clones.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA-01 (AAAB Hybrid)</td>
</tr>
<tr>
<td>Average number of fingers per bunch</td>
</tr>
<tr>
<td>Plant crop</td>
</tr>
<tr>
<td>Farm-gate price (US$ per 1000 fingers)</td>
</tr>
<tr>
<td>Gross income (US$ ha(^{-1}) yr(^{-1}))</td>
</tr>
<tr>
<td>Ratoon crop</td>
</tr>
<tr>
<td>Farm-gate price (US$ per 1000 fingers)</td>
</tr>
<tr>
<td>Gross income (US$ ha(^{-1}) yr(^{-1}))</td>
</tr>
</tbody>
</table>

1 Based on a planting density of 1111.11 plants per ha.
Evaluation of *Musa* germplasm against banana weevil borers

B. Padmanaban, P. Sundararaju, K.C. Velayudhan and S. Sathiamoorthy

Bananas and plantains constitute the fourth most important crop of the developing world and India is the largest producer in the world. Of the 40 million tonnes of fruits produced in India, banana occupies the top position with an annual output of 13.5 M\textsuperscript{T} from an area of 400 000 ha. Among the insect pests of banana, the banana rhizome weevil, *Cosmopolites sordidus* (Germ.) and banana stem weevil, *Olophorus longicollis* (Oliv.) are the key pests limiting the production and productivity of bananas and plantains (Figure 1) (Ostmark 1974). Both types of weevil borers have been a threat to banana under garden land cultivation and their occurrence in non-traditional areas of Tamil Nadu has been reported (Padmanaban and Sundararaju 1999). Bionomics and chemical control of the pests have been studied (Dutt and Matti 1972, Reghunath et al. 1992, Mathew et al. 1997).

The reaction of banana clones to major biotic stresses has been studied by Anitha et al. (1996). This study did not however include the banana pseudostem weevil *O. longicollis*. Indeed, only a limited number of banana cultivars have been evaluated against banana stem weevil (Charles et al. 1996). This paper reports on field screening of diverse *Musa* germplasm under natural infestation of banana weevil borers: banana stem weevil (BSW, *O. longicollis*), and banana rhizome weevil (BRW, *Cosmopolites sordidus*).

A negative correlation between corm hardness and infestation rate led to the hypothesis of mechanical resistance to oviposition or larval development of the rhizome weevil (Pavis and Minost 1993). Ortiz et al. (1995) indicated that investigations of resistance mechanisms in the corm should consider presence of antifeedants or absence of essential nutrients. Attraction of the pseudostem to adults was not used as a criterion of resistance to weevil be-

References


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cause no correlation was found between attraction and infestation (Pavis and Minost 1993).

Materials and methods
Banana germplasm available at the National Bureau of Plant Genetic Resources (NB PGR) Regional Station, Vellanikkara, Kerala was evaluated under field conditions against the banana weevil borers during 1999-2000. The crop was raised during 1995 with a row-to-row and plant-to-plant distance of 2.8 x 2.8 m. The normal package of practice was followed and the crop was managed under rainfed conditions. Stem girth near crown as well as the base and percentage of infestation were recorded. High-infested plants were cut open to record the number of adult weevils and grubs inside the plant. From the harvested plants the rhizome was uprooted and the damage assessed.

Results and discussions
Field evaluation of banana germplasm against banana stem weevil indicated that out of the 229 accessions, 62 accessions belonging to AAB, ABB, AA, BB and ABBB genomic groups had BSW infestation. Maximum infestation was noticed in the AAB genome, and 37 accessions belonging to the following genome: ABB, AAB, AA, BB, AB, AAA, AABB and AAAA were found free from BSW infestation (Table 1).

<table>
<thead>
<tr>
<th>IC No.</th>
<th>Local name</th>
<th>Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR 7</td>
<td>Sannachendakali</td>
<td>AA</td>
</tr>
<tr>
<td>84809</td>
<td>Karumpooovan</td>
<td>AAB</td>
</tr>
<tr>
<td>TCR 22</td>
<td>Nattuwazhai</td>
<td>ABB</td>
</tr>
<tr>
<td>TCR 29</td>
<td>Siukostu</td>
<td>ABB</td>
</tr>
<tr>
<td>84833</td>
<td>Sakkai (Chakkiya)</td>
<td>ABB</td>
</tr>
<tr>
<td>84863</td>
<td>Pozhchendu</td>
<td>AAB</td>
</tr>
<tr>
<td>84889</td>
<td>Senkadi</td>
<td>AAA</td>
</tr>
<tr>
<td>TCR 78</td>
<td>Koombilakai</td>
<td>AAB</td>
</tr>
<tr>
<td>TCR 133</td>
<td>Moris</td>
<td>AAA</td>
</tr>
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<td>127933</td>
<td>Kidali</td>
<td>AA</td>
</tr>
<tr>
<td>127936</td>
<td>Tongat</td>
<td>AA</td>
</tr>
<tr>
<td>127938</td>
<td>Namrai</td>
<td>AAB</td>
</tr>
<tr>
<td>127940</td>
<td>Sannachendakali</td>
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</tr>
<tr>
<td>127941</td>
<td>Karivazha</td>
<td>AAA</td>
</tr>
<tr>
<td>127943</td>
<td>Bodles Alta Fort</td>
<td>AAAA</td>
</tr>
<tr>
<td>127944</td>
<td>Hybrid sawi</td>
<td>ABBB</td>
</tr>
<tr>
<td>127945</td>
<td>Elavazha</td>
<td>BB</td>
</tr>
<tr>
<td>127947</td>
<td>Kunnan</td>
<td>AB</td>
</tr>
<tr>
<td>127952</td>
<td>Padalmoongil</td>
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</tr>
<tr>
<td>127958</td>
<td>Radjasree</td>
<td>AAB</td>
</tr>
<tr>
<td>127963</td>
<td>Vannan</td>
<td>AAB</td>
</tr>
<tr>
<td>127974</td>
<td>Karibale</td>
<td>AAB</td>
</tr>
<tr>
<td>127978</td>
<td>Velipadathi</td>
<td>AAB</td>
</tr>
<tr>
<td>127980</td>
<td>Peyan</td>
<td>AB</td>
</tr>
<tr>
<td>127981</td>
<td>Ashy Bathesa</td>
<td>ABB</td>
</tr>
<tr>
<td>127984</td>
<td>Octoman</td>
<td>ABB</td>
</tr>
<tr>
<td>127986</td>
<td>Kalibow</td>
<td>ABB</td>
</tr>
<tr>
<td>127987</td>
<td>Boodithabontha bath</td>
<td>ABB</td>
</tr>
<tr>
<td>TCR 195</td>
<td>Padathi</td>
<td>AAB</td>
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<td>Ennabinen</td>
<td>ABBB</td>
</tr>
<tr>
<td>127996</td>
<td>Cheenabale</td>
<td>AAB</td>
</tr>
<tr>
<td>TCR 216</td>
<td>Boonihale</td>
<td>ABB</td>
</tr>
<tr>
<td>TCR 221</td>
<td>Morei</td>
<td>AAA</td>
</tr>
<tr>
<td>TCR 241</td>
<td>Padalmoongil</td>
<td>AB</td>
</tr>
<tr>
<td>84776</td>
<td>Njilipooovan</td>
<td>AB</td>
</tr>
<tr>
<td>84760</td>
<td>Madavazha</td>
<td>ABB</td>
</tr>
<tr>
<td>TCR 300</td>
<td>M. balbisana</td>
<td>BB</td>
</tr>
</tbody>
</table>

Incidence of BSW was recorded in 5.50% of the accessions in 1999 and there was a four-fold increase (21.36%) during 2000.

Due to the BSW infestation, there was 50-86% reduction in the stem girth near the crown region. In the infested plants, 2-15 adult weevils, 10-15 grubs and 5-8 pupal cases were found. Dutt and Maiti (1972) have reported that the portions of the banana pseudostem with circumference ranging from 25 to 30 cm and up to a height of 125 cm in tall varieties like Mantam (AAB), Champa (AAB) and Kanchekele (AAB), and up to a height of 100 cm in dwarf varieties like Kabuli (AAA), are the preferred sites for oviposition. Our studies indicated that there was no relation between infestation, stem girth and plant height, with even smaller plants being infested.

Field evaluation of banana germplasm against banana rhizome weevil, C. sordidus, was carried out on 143 accessions.

<table>
<thead>
<tr>
<th>IC No.</th>
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</tr>
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<tbody>
<tr>
<td>TCR 7</td>
<td>Sannachendakali</td>
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<td>Karumpooovan</td>
<td>AAB</td>
</tr>
<tr>
<td>84863</td>
<td>Pozhchendu</td>
<td>AAB</td>
</tr>
<tr>
<td>84866</td>
<td>Sakkai</td>
<td>AB</td>
</tr>
<tr>
<td>84889</td>
<td>Senkadi</td>
<td>AAA</td>
</tr>
<tr>
<td>127949</td>
<td>Njilipooovan</td>
<td>AB</td>
</tr>
<tr>
<td>TCR 216</td>
<td>Borthibale</td>
<td>ABB</td>
</tr>
<tr>
<td>TCR 261</td>
<td>Njilipooovan</td>
<td>AB</td>
</tr>
</tbody>
</table>

Anitha et al. (1996) reported that 87 clones representing various genomic groups (AA, AB, AAA, AAB and ABB) were screened under natural conditions. Among the AA clones, Sannachendakali was tolerant to rhizome weevil. In the AB group, Njilipooovan, Kunnan and Poomkalli were resistant to the pest. Among AAB clones, Msoore Poovan was highly tolerant to rhizome weevil attacks. In the ABB group, Jamani was tolerant to the pest, while Malamolthan and Peykunnan have moderate levels of resistance to BSW.

Host plant resistance introduced through breeding offers a safe and long-term control strategy for the banana weevil (Seshu Reddy and Lubega 1998). Observations on the germplasm evaluated against the banana weevil borers indicate that weevils have a preference for AAB and ABB genomic groups. Studies conducted by various authors elsewhere also indicated a similar trend (Haddad et al. 1979, Mesquita et al. 1984, CRBP 1992, Simmonds 1966).

The BSW shows a high degree of host plant preference. When all the commercial cultivars like Nendran, Robusta, Rasthali, Red Banana, Pisang Awak are available in the same vicinity, the stem weevil recognizes and infests only plantain cultivars. The ability of the weevil to distinguish an acceptable host plant from others may be aided by the presence of an array of sensory chemoreceptors on the antenna and mouth parts (Nahif et al. 1994, Nahif et al. 2000). Further studies are needed at the laboratory level with the identified resistant accessions to single out the most promising.

Acknowledgements
Thanks are due to Dr Z. Abraham, Scientist-in-charge, NB PGR, Thrissur for providing necessary facilities and Ms C. Rajalakshmy, Technical Officer, for technical assistance.

References
Mathew M.P., S.B. Nair & S. Sivaraman. 1997. Management of pseudostem borer of banana, Oikoporo...
Distribution of Fusarium wilt of banana in Kenya and its impact on smallholder farmers

J.N. Kung’u, M.A. Rutherford and P. Jeffries

Banana (Mus a spp.) is becoming an important crop in the Kenyan economy, which is predominantly agriculture-based. For the last 20 years banana production has increased significantly, partly due to a reduction in income from coffee, which has forced farmers to adopt banana as an alternative cash crop for local markets. Bananas are mainly grown by smallholder farmers, and the crop integrates well into other agricultural enterprises. For instance, harvested pseudostems are an important fodder for dairy animals, particularly during drought, thus contributing to milk production (another important source of income to smallholder farmers) and manure which is recycled back to the farm. Although the crop has potential as an export commodity for Kenya, banana diseases and pests are major limiting factors in its production (Kung’u 1995). Fusarium wilt is currently the most important banana disease in Kenya (Kung’u 1995, 1998).

The effects of climate and rainfall on distribution, incidence and severity of Fusarium wilt of banana are complex (Wardlaw 1972). In studying the effects of climate, Wardlaw (1972) suggested that the effects of soil type should also be considered. Earlier observations showed that the spread of Fusarium wilt in Central America was faster in some regions than in others (Stotzky and Martin 1963), leading to the classification of soils in banana areas of the region on the basis of “effective banana-producing life” (short, intermediate and long). Subsequently, attempts were made to correlate effective banana-producing life with specific soil properties such as texture, pH, cation-exchange capacity, total soluble salts, available nutrients, organic matter and drainage (Stotzky and Martin 1963). Among these properties clay mineralogy was found to correlate very closely with the effective banana-producing life.

Kenya has a diverse ecology, being partitioned into a number of agroecological zones (AEZs) (Jaetzold and Schmidt 1982a, b, c) and agroclimatic zones (ACZs) (Sombroek et al. 1982) within which there are also diverse soil types. It is of importance to consider either or both of these zones when studying plant disease epidemics. AEZs were established by FAO (1978) based on climatic yield potential of the main leading crops within a region. ACZs on the other hand were based on moisture availability and average annual temperature of a region (Sombroek et al. 1982). A moisture availability zone of an ACZ was determined as the ratio of measured annual rainfall (r) and computed average annual evaporation (Eo).

The objectives of this study were to determine the distribution of Fusarium wilt in Kenya, identify cultivars affected by the disease and determine whether there is any apparent correlation between disease distribution and AEZ, ACZ or specific soil factors. This information is of importance to disease management, as it will facilitate deployment of tolerant/resistant cultivars in disease epidemic zones, while susceptible cultivars (which currently cannot be replaced by other banana types) may only be grown in ‘non-epidemic’ or disease-free zones.

Materials and methods

Survey areas where samples were collected

Areas surveyed covered all major banana-growing districts of Kenya except Lamu district. The survey area was divided into three main regions (Figure 1), the Coast area (Kilifi, Kwale and Taita-Taveta districts), the Central/Eastern area (Murang’a, Kirinyaga, Nyeri, Embu and Meru districts) and the Western area (Kisii, Nandi and Trans-Nzoia districts).
Homa Bay, Migori, Kisumu, Siaya, Kakamega and Busia districts). Farms and plants to be sampled were taken at random (Kung’u 1998).

Isolation and identification of *F. oxysporum f.sp. cubense* (Foc) strains

On return to the laboratory, individual leaf-sheaths were peeled away from the pseudostem pieces collected from the field, the outer surface wiped with 70% (v:v) alcohol and the material briefly flamed. Using a sterile scalpel, small pieces of discoloured vascular bundles were excised from the flamed leaf sheaths and placed onto 2% (w:v) tap water agar (TWA). Emerging fungal colonies were subcultured onto potato sucrose agar (PSA) and synthetic nutrient agar (SNA Nirenberg 1976). Isolates were identified to species level based on morphological keys described by Booth (1971) and Nelson et al. (1983). Monoconidial cultures were raised from the SNA cultures for further characterization and preserved in sterile soil (Smith and Onions 1983) for the duration of the study. Confirmation of Foc was through pathogenicity tests as detailed earlier (Kung’u 1998).

Data processing and mapping

Maps of the area surveyed were prepared from a base map of Kenya (scale 1:1000 000) using MapInfo for Professionals™ (Version 4.1). Similarly, maps for ACZ, AEZ, soil types and elevation were also prepared from the base maps. Data on the geographical origin of isolates and host cultivars were geocoded (assigned geographic coordinates) to provide accurate maps of Fusarium wilt distribution within the study areas. These disease distribution maps were then overlaid onto AEZ, ACZ, soil and elevation maps of the study areas to create thematic maps depicting disease distribution in relation to cultivar, AEZ, ACZ, soil type (including physicochemical properties) and elevation.

Results and discussion

Distribution of Fusarium wilt and cultivars affected

Fusarium wilt of banana was observed in all of the districts surveyed with the exception of Nyeri district (Figure 1).

Coast survey area

In Kwale and Kilifi districts bananas are grown along the coastal strip. Between Vanga in the South and Malindi in the North the predominant cultivar, Bluggoee (ABB), was found to be affected by Fusarium wilt. Tall and dwarf Bluggoee types were grown, the former being the most common, and both exhibited symptoms. It is suspected that this cultivar has gradually been decimated by a number of factors, including Fusarium wilt, that had not been recognized by farmers. Cultivars Wang’ae (=Ney Poovan, AB), Mhale (AA) and Mbuu (probably silk, AA), which are not cultivated by many farmers in the area, were also affected. Dwarf Cavendish (AAA) was also commonly grown but was not found to be affected by the disease even on farms where it was intercropped with severely affected Bluggoee plants. Gros Michel (AAA) was not found in the coastal strip.

Central and Eastern survey areas

In the Central and Eastern areas Gros Michel (AAA), Wang’ae (AB), Muraru (AA’), Mbuu (ABB) and Mugithi (ploidy unknown) were found to be affected by Fusarium wilt. Muraru, probably an East African Highland banana (EA-AAA), seemed to be tolerant of the disease, and was only found to be affected on some farms in Murang’a district. Gros Michel, mostly grown in Murang’a district but also found in significant numbers in Embu, Kirinyaga and Meru districts, was also affected. It was not common in Nyeri district where, despite intensive surveys, wilt was not observed (including on Wang’ae). The extent of wilt has forced some farmers in Murang’a and Embu districts to completely replace Gros Michel with Lacatan (AA).

Fusarium wilt was not found on any of the Cavendish bananas types (e.g. Lacatan and Valery) grown in the Central or Eastern areas, nor on East African Highland bananas (EA-AAA) such as Kiganda, Mutika and Mutore or Mutahato (probably also AAA).
Table 1. Number of plant samples per susceptible cultivar collected in districts surveyed for Fusarium wilt in Kenya (numbers include only those samples from which isolates tentatively identified as *F. oxysporum* were obtained).

<table>
<thead>
<tr>
<th>District</th>
<th>Blugge</th>
<th>Gros Michel</th>
<th>Muraru</th>
<th>Wang’ae</th>
<th>Mbuu</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwale</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Kilifi</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>T/Kakata</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Murang’a</td>
<td>6</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Kin’ya</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Meru</td>
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<td>7</td>
<td>4</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Embu</td>
<td>13</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Kisii</td>
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<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Migori</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Homa Bay</td>
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<td>0</td>
<td>11</td>
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<td>0</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Busia</td>
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<td>3</td>
<td>4</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Kakamega</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>39</strong></td>
<td><strong>38</strong></td>
<td><strong>93</strong></td>
<td><strong>17</strong></td>
<td><strong>194</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Western survey area**

Fusarium wilt was found in all districts surveyed in the Western survey area. In Kisii district, the disease was restricted to cultivar Wang’ae (known locally as Egesukari). Other cultivars in Kisii, which were mainly East African Highland types, showed no symptoms of wilt. Farmers interviewed in the district stated that they had generally noticed a drastic decline in Wang’ae production due to a disease that they did not understand but that, from their descriptions, appears to be Fusarium wilt. Incidence of the disease was almost 100% on some farms where pure stands of Wang’ae were grown.

In Homa Bay and Migori districts Fusarium wilt was found on Wang’ae and Mbuu (Odhigo), the predominant cultivars in the area, and on Blugge, Wang’ae, and Mbuu (Odhigo) in Kismu, Siaya and Busia in Kakamega district the disease was found primarily on Wang’ae. Although only a few Blugge plants were observed in Kakamega district, these were disease-free.

Isolation and identification of *F. oxysporum* strains from symptomatic plant material

A total of 204 samples were collected from the three survey areas, of which 194 yielded isolates that were identified as *Fusarium oxysporum* (Table 1).

**Correlation between Fusarium wilt distribution and ecological factors**

From the survey observations, the distribution of Fusarium wilt appears to correlate with ACZ, and specifically temperature zone (Figures 2 and 3), but not with AEZ or soil type. The disease was generally restricted to temperature zones 1, 2, and 3 (0-1500 m asl) but was most prevalent in temperature zone 3 (20-22°C, 1200 to 1500 m asl). Temperature zone 1 (24-30°C) is the predominant zone in the coastal strip, except at Taita hills (temperature zone 2). The coast region of Kenya generally has hot and humid conditions, which do seem to favour development of the disease. In the Central and Eastern Provinces the disease was also found in temperature zone 2 (22-24°C, 900-1200 m asl). The temperature zones 1, 2, and 3 are also characterized by at least occasional, if not frequent, water stress, particularly during the months of July, August and September. It is during these periods that bananas appear to be worst affected by Fusarium wilt, either because infestation is more likely and more rapid or simply because symptoms are more pronounced. Wilt
was not observed in temperature zones 4 (18–20°C), 5 (16-18°C), or 6 (14-16°C) where bananas also grow fairly well.

There are many reasons, direct and indirect, why temperature may have an effect of wilt development. These include the ability of the host plant to produce gels and tyloses that help to occlude vascular vessels and hence restrict movement of the pathogen within them. At temperatures most conducive to disease development (27-28°C), for example, gel structures in susceptible hosts are much weaker than in resistant hosts, thus permitting systemic colonization in the former (Beckman 1990). Although the mean annual temperature for zone 3 for example is 22-24°C, the maximum temperature for this zone during the months of December to March ranges between 26.4-30.4°C which is within the temperatures conducive to disease development in susceptible hosts.

In addition to the absence of disease on the East African Highland bananas, the disease was not found on the Cavendish group either. The Cavendish is obviously a suitable and available alternative to the susceptible dessert bananas.

Although precise dates are unavailable, it is known that Bluggoe and Wanga were introduced into Kenya earlier than Gros Michel. Given that the first report of Fusarium wilt in Kenya was in the Coast Province (possibly on Bluggoe) in 1952 and in the same year in Central Province (possibly on Wanga), it can be speculated that Fusarium wilt of banana originated with these cultivars in Kenya.

Impact of the disease on the livelihoods of unemployed smallholder farmers

The impact of Fusarium wilt of banana in Kenya is considerable. At present it directly affects the livelihood of more than one million people in the Coastal survey area, more than three million in the Central and Eastern survey areas and more than over five and a half million in the Western survey area (figures based on 1989 Kenya’s population census and not including consumers of bananas based in urban centres and other areas). Since the first report in 1952, the status of the disease in Kenya was unclear for nearly 40 years. The immense socioeconomic impact resulting from the devastating disease outbreaks in the 1980s, particularly in the Central and Eastern regions, was probably based largely on the declining status of coffee as a major source of income for smallholder farmers. From the 1960s to the middle of the 1970s, coffee production on smallholder farms provided farmers with a substantial income (Turner et al. 1997). Farmers increased coffee production areas at the expense of other crops as coffee could generate sufficient income to enable them to meet all of their basic necessities, including food purchase. During this period coffee was the major earner of foreign exchange for Kenya and was referred to as ‘black gold’. However, the 1978-1979 global oil crisis caused a significant increase in the price of petroleum and led to a rise in the cost of coffee production due to increased costs of oil-dependent inputs. Between 1980 and 1990, real international prices for Africa’s coffee exports fell by 70% (Turner et al. 1997). Earnings from coffee production fell far below those for food crops such as bananas, maize, and beans, and, by 1986, most farmers had abandoned coffee as a crop and switched to banana production. Dessert cultivars, such as the wilt susceptible Gros Michel, Wanga, and Muraru, were the most popular in urban centre markets and were planted en masse. Without nurseries to obtain suckers, farmers multiplied plantlets from existing material and in this way no doubt exacerbated problems caused by wilt. Gros Michel, locally known as ‘Kampala’ in some regions, was initially introduced by a few farmers as a few suckers from a neighbouring country in the late 1960s. From this original source the cultivar, which has a very narrow genetic base, has gradually been distributed throughout many of the main banana-growing regions, particularly in Central and Eastern Kenya. Although the imported material may have been pathogen-free, distribution of highly susceptible planting material in this way may well explain the rapid, widespread and devastating spread.

Figure 3. Correlation between Fusarium wilt distribution and agroclimatic zones in the Western region of Kenya (the Roman numerals represent the moisture zones while the Arabic numbers indicate the temperature zones). The disease was restricted within the temperature zone 3.
Vegetative compatibility groups of the populations of Fusarium oxysporum f.sp. cubense in Vietnam

Do Nang Vinh, Nguyen Van Khiem, Chu Ba Phuc and Le Huy Ham

Fusarium wilt (Panama wilt) disease caused by Fusarium oxysporum Schlecht. fsp. cubense (E.F. Smith) WC. Snyder & H.N. Hans (Snyder et al. 1940), is regarded as one of the most significant threats to banana (Musa spp.) production worldwide (Persley et al. 1987), including Vietnam (Akkil 1968).

Fusarium oxysporum f.sp. cubense (Foc) affects species of Musa and Heliconia, and strains have been classified into four physiological races based on their pathogenicity to host cultivars: race 1: Gros Michel (AA), Lady Finger (AAB); race 2: Bluggoe and closely related clones (ABB); race 3: Heliconia sp.; and race 4: Cavendish cultivars and all cultivars susceptible to race 1 and race 2 (Persley et al. 1987).

Vegetative compatibility groups (VCGs) are a natural way of subdividing fungal populations. The exchange of genetic information in an asexual population is limited to the individuals that can form a viable heterokaryon. The loci that govern heterokaryon incompatibility are referred to as het, tal, vc and vic (Leslie 1990). The het loci behave as if they were part of a recognition system that enables individuals to identify each other and to differentiate themselves from each other. The het loci can delimit the pathotypes of assexual phytopathogenic fungi, as occurs in the genus Fusarium (Correll et al. 1987, Ploetz 1990).

Our objective in this study was to characterize isolates from different provinces of North Vietnam using vegetative compatibility.

Material and methods
In order to determine the VCGs to which belong the Vietnamese Foc populations, banana plants bearing symptoms of Fusarium wilt were sampled in different provinces in North Vietnam.

Spores isolated from discoloured vascular strands dissected out of banana plants affected by Fusarium wilt were maintained on sterile filter paper as described by Correll et al. (1986). Nitrate non-utilizing (nit) mutants were generated by transferring pieces of colonized filter paper onto potato dextrose agar (PDA) amended with 1.5% KClO₃ and incubating for 7-14 days at 25°C. Chlorate-resistant mutants were transferred to minimal medium (Puhalla 1995) and were assigned to phenotypic classes as described by Correll et al. (1987). Any nit I or nit 3 mutants were paired with tester nit M mutants of four known VCGs (VCG 0123, 0124, 0124/5 and 0125) on minimal media with nitrate as the sole nitrogen source. The development of dense aerial mycelium at the point of contact of the two nit mutants was an indication of complementation.

Results and discussion
Forty-two isolates of Foc were collected and tested from 11 districts of

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7 provinces in North Vietnam (Hanoi, Hatay, Hungyen, Vinhphuc, Phthuo, Bacnin, Thuthinhue). Twenty-one isolates from all seven provinces belonged to VCG 0124; 4 isolates from Hanoi and Hungyen provinces belonged to VCG 0124/5; 2 isolates from Hanoi and Hungyen provinces belonged to VCG 0125; 2 isolates from Hungyen province were vegetatively compatible with both VCG 0124/5 and 0125; 13 isolates from Hanoi, Hungyen and Bacnin provinces were vegetatively compatible with VGCs 0124, 0124/5 and 0125. All of these VCG isolates are in race 1.

Isolates that were cross-compatible were recovered in this study, forming a bridge between VGCs 0124, 0124/5, and 0125. They may represent a stage in the divergence and formation of the new VCG. Similar results were obtained with isolates studied in Australia by Brake et al. (1990).

Results of analysis showed that VCG 0124 and "VCG 0124-0124/5-0125" were widespread, being detected in North Vietnam. No isolate was identified as belonging to VCG 0123 in North Vietnam, whereas Mai Van Tri (1997) collected and analyzed 8 isolates from 6 districts of 4 provinces in South Vietnam and showed that 5 isolates belonged to VCG 0123, and 3 isolates belonged to VCG 0124/5. This can indicate that VCG 0123 and 0124/5 were widespread in South Vietnam. In 1998, Bentley et al. analyzed 21 isolates from 7 provinces in North, Centre, and South Vietnam. They showed that 5 isolates belong to VCG 0124/5, 11 isolates belong to VCG 0123, and 5 isolates belong to VCG 0124/0125.

Results of analysis indicated that Chouei Tay (Pisang Awak ABB), Chouei Ngop (Bluggoe ABB) and Chouei Com La (Silk ABB) were attacked by _Foc_ race 1 (VCGs 0124, 0124/5, 0125; VCG 0124/5-0125, VCG 0124-0124/5-0125). Infection by race 4 on the Cavendish group (AAA) has not yet been detected in Vietnam.

This study demonstrates the value of using vegetative compatibility analysis to assess variability in populations of _Foc_. It also gives an indication of the dissemination potential of strains of this pathogen and contributes to the more effective deployment of resistant banana cultivars.

It is important to select and create new cultivars with resistance to _Foc_ to replace infected Chouei Tay and Com La. It may be possible to use Cavendish cultivars to replant these varieties with _Foc_ is present. The results of our study also indicate that it is essential to apply quarantine procedures to prevent race 4 from being introduced to Vietnam from other countries.

Acknowledgements
The authors wish to acknowledge the financial support of the World Bank for this study. We are also grateful to De Natalie Moore, Mr Ken Pegg and Mr Bob Davis, QDPI (Department of Primary Industries, Queensland, Australia) who supervised the VCG techniques and provided the testers.

References


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Country report

**Black Sigatoka disease (Mycosphaerella fijiensis Morelet) in Mexico**

Black Sigatoka, caused by the ascomycete fungus _Mycosphaerella fijiensis_ Morelet (teleomorphic) or _Paracercospora fijiensis_ (Morelet) (Deighton, anamorphic), is the most serious disease affecting the commercial production of banana and plantain ( _Musa_ spp.) in most of the production regions in the world (Fullerton 1994, Fullerton and Stover 1990, Mourichon and Fullerton 1990). Black Sigatoka was identified in America for the first time in 1972 (Stover and Dickson 1976) in Honduras, from where it spread to all the countries in Central America, to Mexico and to part of South America (Fullerton and Stover 1990, Stover 1980). In Mexico, it was first identified in the south-east part of the country in Chiapas and Tabasco states (Contreras 1983) and it is currently found in all the banana and plantain production regions (Orozco-Santos 1998).

The presence of the disease in Mexico has caused serious losses in all the banana production regions by disturbing plantation management, in particular with regard to fungicide spray programmes. This has resulted in increased production costs. Today, control of the disease in Mexican plantations depends mainly on chemicals whose effects are completed by certain cultural practices. The work presented here is aimed at providing information about the current situation with regard to the disease in the banana and plant-
taint production regions in Mexico and to review various aspects of epidemiology, treatments and research.

The importance of bananas in Mexico
Banana and plantain are grown in Mexico on 72,700 ha with production of 2.2 million tonnes of fruits, 95% of which is for the domestic market (Orozco-Romero et al. 1998). The production zones are located in the tropical regions around the Gulf of Mexico and on the Pacific coast. The main producer states are Chiapas, Veracruz, Tabasco, Nayarit, Colima, Michoacan, Oaxaca, Jalisco and Guerrero. These form three main production regions: the Gulf of Mexico with 46% of the cultivated area, Central Pacific with 24.4% and Southern Pacific with 30.1% (Figure 1). The taxonomic groups most strongly represented in Mexico are AAA (‘Gran Enano’ and ‘Valery’, Cavendish subgroup), AAB (‘Macho’ or ‘False Horn’ and ‘Dominico’, Plantain subgroup), ABB (‘Manzano’ or ‘Silk’), ABB (‘Pera’ or ‘Cuadrado’) and AA (‘Ditiil’). Information about production regions, taxonomic groups and cultivated areas in Mexico is grouped in Table 1.

The main climate and elevation characteristics of the production regions in Mexico are shown in Table 2.

The distribution of black Sigatoka in America
For many years, the disease called ‘chamusco’ or yellow Sigatoka, caused by the fungus Mycosphaerella musicola Leach, was the most serious disease of banana and plantain leaves in Mexico. It first arrived in 1936 in the south-eastern states (Chiapas and Tabasco) and then spread to all the other production regions in the country (Stover 1962). It is now being pushed back by black Sigatoka. This is probably the result of the greater aggression and better adaptability of M. fijiensis in tropical regions whose elevation does not exceed 500 m, as reported by Mouliom-Pefoura and Mourichon (1990) and by Mouliom-Pefoura et al. (1996). The first official notifications of outbreaks of M. fijiensis were made in Chiapas and Tabasco states in 1981. However, the disease had been observed for the first time in the Tapachula zone (Chiapas) at the end of 1980 (Contreras 1983). Black Sigatoka has since continued to spread rapidly and reached Veracruz and Oaxaca states in 1985 (Robles et al. 1988).

In the Central Pacific region, the disease was first detected in Colima state in 1989 and a year later had reached neighbouring Michoacan, Jalisco and Guerrero states. The disease was observed in Nayarit state in November 1994 (Orozco-Santos et al. 1996). This makes it possible to affirm that black Sigatoka is now found in practically all the Musaeeae production zones in Mexico (Orozco-Santos 1998).

Impacts of the disease and chemical control
Black Sigatoka has had a devastating effect on the banana zones of Mexico. The first epidemic caused losses of 50 to 100% of fruit production and a considerable decrease in cultivated areas. The disease caused the disappearance of some 2000 ha of bananas in Tabasco state in the early 1980s. In Colima state, where it was detected eight months later in September 1989, over 3000 ha of plantations were grubbed up for reasons of non-productivity and the losses were estimated to be 50,000 tonnes of fruits. Abandoned areas totalled 5000 ha in March 1991, a 50% decrease in the cultivated area (Orozco-Santos et al. 1996). Today, only 4700 ha is cultivated in Colima state (Orozco-Romero et al. 1998).

The appearance of black Sigatoka in Mexico has resulted in changes in plantation management and especially in fungicide spray programmes. Prior to the 1980s, yellow Sigatoka was the most serious phytosanitary problem for the foliage of cultivated species but did not require strict fungicide spray programmes. The arrival of black Sigatoka considerably changed the control programmes, requiring the use of more powerful fungicides at shorter intervals. It is estimated that measures to control Black Sigatoka represent 35 to 45% of total production costs. In parallel, changes involved greater cultural technical skills (mineral nutrition, population density, deleafing, desuckering, pest, disease and weed control), improving fruit quality and per-hectare yields (Orozco-Santos 1998).

Today, chemical methods form the most reliable alternative for controlling the disease. However, this has increased production costs and has also led to problems of environmental pollution, public health and resistance to fungicides caused by residues of chemicals and protective substances (citroline). Nearly 370 million pesos (US$ 43 million) is spent in Mexico each year on the control of black Sigatoka. Some 430,000 kg of active substances was applied until 1995, consisting mainly of systemic fungicides, and nearly 13 million litres of citroline, i.e. an average of 184 litres per hectare per year. Today, control programmes using protective fungicides have significantly reduced the amounts of citroline or spray oil. Nevertheless, the quantity of fungicide active ingredients per unit area has increased and the total has reached 7 million kg per year in Mexico as a whole (Orozco-Santos 1998).

Little research has yet been conducted on the environmental impact and the health problems resulting from the continuous application of fungicides and citroline in banana plantations. However, we know that certain fungicides and bactericides are highly toxic and act as molecular inducers of the cellular activity responsible for the endocrine functions that regulate the hormonal control of reproduction, sex differentiation and the proliferation of immuno-competent cells (Chambers and Yarbrough 1982). Both human beings and fauna are exposed to fung-
cides and bactericides as a result of aerial spraying, contaminated foodstuffs and polluted potable water supplies. Aerial spraying is certainly a rapid technique for applying chemicals to very large areas. However, water running from storage sites, runways and sprayed zones can pollute the neighbouring aquatic and land environments (Henríques et al. 1997).

The fungicide propiconazole was used in Mexico for 20 years to control black Sigatoka and high concentrations are found in drainage water next to banana plantations, as demonstrated in Costa Rica, where concentration of 24.2 µg per L water have been observed (Mortensen et al. 1998). Mancozeb became the key fungicide in control programmes from 1995 onwards. In Costa Rica, measurement in channels after one spray revealed mancozeb residues ranging from 0.77 to 2.38 µg/cm² (Mortensen et al. 1998). Chlorothalonil has been found to be toxic for aquatic invertebrates and fish, while mancozeb is carcinogenic and benomyl is teratogenic (Lacher et al. 1997).

Furthermore, the intensive use of several systemic fungicides has resulted in the appearance of resistance in the fungus *M. fijiensis* (Castro et al. 1995, Romero and Sutton 1997, 1998). This is because some types of systemic fungicides (benzimidazoles and triazoles) display strong activity at small doses and act on a single site in the pathogen (Russell 1995). Problems of resistance mean that the control of black Sigatoka has become more complex and more expensive since the loss of susceptibility to fungicides means more sprays.

**Table 1. Banana and plantain production regions in Mexico.**

<table>
<thead>
<tr>
<th>Region (states)</th>
<th>Taxonomic groups</th>
<th>Cultivated areas (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulf of Mexico</td>
<td>AAA, AABp*, AA</td>
<td>12 900</td>
</tr>
<tr>
<td>Tabasco</td>
<td>AAA, AABp</td>
<td>14 200</td>
</tr>
<tr>
<td>Veracruz</td>
<td>AAA, AABp</td>
<td>14 200</td>
</tr>
<tr>
<td>Oaxaca</td>
<td>AAA, AABp, AABB</td>
<td>3 900</td>
</tr>
<tr>
<td>Central Pacific</td>
<td>Colima</td>
<td>AAA</td>
</tr>
<tr>
<td></td>
<td>Michoacán</td>
<td>AAA</td>
</tr>
<tr>
<td></td>
<td>Jalisco</td>
<td>AAA</td>
</tr>
<tr>
<td></td>
<td>Nayarit</td>
<td>AAA, AAB, AABp, ABB</td>
</tr>
<tr>
<td>Southern Pacific</td>
<td>Chiapas</td>
<td>AAA, AABp</td>
</tr>
<tr>
<td>Other</td>
<td>AAA, AAB, AABp, ABB</td>
<td>2 500</td>
</tr>
<tr>
<td>National</td>
<td></td>
<td>72 900</td>
</tr>
</tbody>
</table>


**Table 2. Climate and elevation of the production regions in Mexico.**

<table>
<thead>
<tr>
<th>Region</th>
<th>Climate</th>
<th>Temperature</th>
<th>Precipitation (mm)</th>
<th>Number of dry months</th>
<th>Elevation (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulf of Mexico</td>
<td>hot and humid</td>
<td>24-27°C</td>
<td>1700 to 3900</td>
<td>0 to 2</td>
<td>10 to 80</td>
</tr>
<tr>
<td>Central Pacific</td>
<td>hot and dry</td>
<td>26-28°C</td>
<td>700 to 1100</td>
<td>7 to 8</td>
<td>10 to 500</td>
</tr>
<tr>
<td>Southern Pacific</td>
<td>hot and subhumid</td>
<td>26-27°C</td>
<td>1500 to 2500</td>
<td>4 to 5</td>
<td>20 to 80</td>
</tr>
</tbody>
</table>

**Actions undertaken to prevent the spread of the disease**

The presence of black Sigatoka in the banana-growing regions in south-east Mexico led the Dirección General de Sanidad Vegetal to impose permanent domestic quarantine No. 18. The main aim of this measure is to prevent or delay the arrival of the disease in banana zones or regions that are still free of it. Respect of the following points was recommended in the campaign:

1. restriction of movements of plant material from infected zones;
2. the establishment of quarantine stations;
3. forbidding the use of leaves to protect fruits in vehicles during transport;
4. disinfection of vehicles;
5. inspection of banana plantations;
6. application of fungicides;
7. grubbing up the most severely affected plantations.

This quarantine did not have the hoped-for effects and the disease spread throughout Mexico in spite of the long distances (over 1000 km) and the natural barriers (mountain ranges) between banana zones or regions. In only 14 years, the disease spread to all the states in which banana and plantain are grown. The spread of the pathogen can be ascribed to movements of infected plant material (dried foliage) during transport of fruit (Orozco-Santos et al. 1996), of infected plants or rhizomes and wind. *M. fijiensis* ascospores are the main source of inoculation and dispersion for long distances in a given zone (Burt et al. 1997, Stover 1980).

**The behaviour of black Sigatoka**

*Gulf of Mexico*. Several epidemiological studies were performed in the Tabasco region (Avila et al. 1994). Research on the disease is fairly rare in the other Gulf of Mexico production zones (San Rafael, Veracruz and Tuxtepec, Oaxaca). Early symptoms (1 and 2 on Fourné’s scale) in banana plantations with no chemical control appear 18 to 32 days after contamination and leaf spots appear after 34 to 73 days. Full development of the symptoms can take from 30 to 115 days. The longest incubation is seen during the driest period of the year. The disease is endemic and its severity varies with the climatic conditions. The most severe period is during the most rainy weather and reaches 15 to 25% from July to December. The disease is less aggressive from January to March with average severity of 5 to 10% (Ramírez and Rodríguez 1996).

*Central Pacific*. From June to November in banana plantations with no chemical control, the incubation period running from infection to the early stage (2 on Fourné’s scale) lasts for 24 to 39 days and the spot stage (4 on Fourné’s scale) from 33 to 58 days. In the dry season (December to May), incubation time is 48 to 87 days until the early stage and 84 to 141 days until spotting stage. The most serious damage is caused to the most recent leaves. The leaves emitted from June to October are totally destroyed over a period of 82 to 120 days, whereas those emitted from November to May resist for 135 to 200 days. The most marked severity is closely linked with the rainy season (June to October) and that of dew formation on leaves (November to January). These results show that under dry tropical conditions black Sigatoka displays an epidemic phase induced by rainfall and a low-severity phase in the dry season (Orozco-Santos 1998).

*Southern Pacific*. Information gathered in a banana plantation with inadequate chemical control showed that the most serious damage (severity 12 to 25%) occurs from June to December, the season with the heaviest rainfall. During this period, 25 to 58% of infected leaves 4 to 6 are at the spotted stage. The period of least severity (January to May) coincides with that of the lowest rainfall, during which spotting is observed on 7 to 25% of infected leaves 7 to 9 (Escudero, unpublished data).

**Treatment of black Sigatoka**

Treatment of the disease in banana plantations is strongly dependent on fungicides. Their action is completed by a number of cultural practices (deleafing,
desuckering, drainage, weed management and mineral nutrition) aimed at reducing the sources of inoculation and preventing the coinciding of conditions favourable for pathogen development (Marín and Romero 1992). Until 1995, chemical control was performed using systemic fungicides belonging to the triazole group (tebuconazole, propiconazole, bitertanol and hexaconazole), the pyrimidines (fenamidone), the benzimidazoles (benomyl, carbendazim and thiophanate-methyl) and morpholines (tridemorph). The strobilurines (azoxystrobin) and other triazoles (fenbuconazole) have been added more recently (Orozco-Santos 1998). Contact fungicides (chlorotoluron and mancozeb) were also included in the spray programmes. Today, the use of protective fungicides is increasing in all production zones (Escudero and Rendón 1996) with applications at 7 to 12-day intervals.

The traditional systemic fungicide/protective substances programme in the Gulf of Mexico required 20 to 25 sprays in the San Rafael zone in Veracruz and 30 to 35 in the Tabasco zone. During the rainy season, systemic fungicides were used alone or mixed every 10 to 12 days and contact fungicides were sprayed every 14 days during the dry season (Ramírez et Rodríguez 1996). Spray programmes have been introduced recently that consist solely of protective fungicides (mainly mancozeb) to avoid the use of citroline. Spray intervals vary from 7 to 12 days according to the time of year. Protective programmes result in 40 to 52 sprays per year.

In the Central Pacific region, the number of sprays of systemic and protective fungicides varies from 15 to 20. The disease is controlled during the rainy season (June to October) and the dry season (November to January) by spraying systemic fungicides every 14 to 21 days, whereas protective or systemic fungicides are sprayed every 25 to 40 days in the dry season (January to May) (Orozco-Santos 1998, Orozco-Santos et al. 1996). Recent studies have shown that with the help of the biological warning system proposed by Marín and Romero (1992), only 10 to 12 sprays are needed during the rainy and dry seasons and no sprays are necessary during the dry season (Orozco-Santos 1995). Control of the disease has been found to be inadequate in plantations intercropped with coconut. Indeed, the trees mean that the aircraft must be flown at an altitude of 35 to 40 m, causing a loss of part of the emulsion as it is deposited on the palms (Orozco-Santos et al. 1996). In programmes using protective fungicides such as mancozeb, spraying is required weekly during the rainy season and every 10 to 14 days during the dry season, making a total of 30 to 35 sprays during the year.

In the Southern Pacific region, up to 35 sprays were required each year in the traditional systemic fungicide/protective substances programme, with systemics sprayed every 10 to 14 days during the rainy season and an alternation of systemic and protective fungicides during the dry season. In this region, as around the Gulf of Mexico, only protective fungicides are used (mainly chlorotoluron) (Escudero and Rendón 1996). Applications are performed weekly during the rainy season and every 10 to 14 days during the dry season.

At the world scale, chemical control of black Sigatoka is considered to be a high-risk activity because of the problems of the resistance developed by the fungus to several fungicide groups. There have been numerous publications on the loss of susceptibility of M. fijiensis to benzimidazoles (Romero and Sutton 1998, Stover 1979) and more recently to triazoles (Castro et al. 1995, Romero and Marín 1990, Romero and Sutton 1997). The evaluation of new fungicide substances with little or no harmful effects on the environment and health is becoming a priority in the search for new ways of managing the disease. This group includes azoxystrobin, which is safe from the environmental point of view. In addition, a new substance called acibenzolar-S-methyl has come on to the market (Madrigal et al. 1998); this is reported to activate natural plant defences, a phenomenon known as acquired systemic resistance (Sticher et al. 1997). A small number of systemic fungicides are used in the control of black Sigatoka today and it is urgent that they should be used properly to ensure them a longer useful life and maintain their efficacy with regard to the fungus (Marín and Romero 1992, Stover 1990, Welemaker 1990).

**Research on black Sigatoka in Mexico**

Research on the disease has been oriented towards the biology of the pathogen, epidemiology, the evaluation of plant material, chemical control, biological warning and, more recently, resistance to fungicides, genetic diversity of the pathogen and genetic transformation, the latter being carried out outside of the banana and plantain production zones (Table 3).

**Conclusions and prospects**

Since its appearance in Mexico in 1980, black Sigatoka has become the main phytosanitary problem in all the banana and plantain production zones. The disease has adapted to various environmental conditions and the pathogen has become more aggressive, making farming more difficult and increasing production costs. Incidence is less marked in the dry tropical region (Central Pacific) than in the humid tropical regions (Gulf of Mexico and Southern Pacific) because of differences in precipitation depth and distribution. In 20 years, the disease has spread to all the banana production zones, where chemical control is the most commonly used response. However, as time passes, it seems that fungicide application is not a suitable solution because of the complex nature of the pathogen (type of reproduction, pathogenicity and spread among other features) and the host characteristics (genetic uniformity, extensive planting, etc), which have facilitated a very close host-para site relationship. Research should be concentrated on sustainable management of the disease aimed at reducing the risks of environmental pollution and of danger to health and enable the conservation of natural resources. The evaluation of genetic material with re-

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**Table 3. Lines of research on black Sigatoka in Mexico.**

<table>
<thead>
<tr>
<th>Lines of research</th>
<th>Gulf of Mexico (Tabasco)</th>
<th>Central Pacific (Tolima)</th>
<th>Southern Pacific (Chiapas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biology of the fungus</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cultural practices</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chemical control</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Biological warning</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological control</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Evaluation of germplasm</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Susceptibility to fungicides</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Genetic diversity</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

*Studies performed by the Centro de Investigaciones Científicas de Yucatán (A. James, personal communication), Universidad de Colima and the Instituto Nacional de Investigaciones Forestales, Agropecuarias y Pesqueras. Note: The genetic transformation studies are carried out by the Centro de Investigaciones Avanzadas (CINVESTAV) of the Instituto Politécnico Nacional (Gómez-Lim 1998).*
sistance to the disease (Orozco-Romero et al. 1998) and genetic transformation (Goméz-Lim 1998) are priority challenges to be taken up in the medium and long term in the research programme on Musaceae in Mexico. In the short term, it is important to continue research on Cavendish subgroup bananas (‘Gran Enano’ and ‘Valery’) and on plantain cultivars in order to improve management of the disease. Studies on cultural control, biological warning and the evaluation of fungicide spray programmes according to their impact on the environment will make it possible to reduce the number of spray cycles. In parallel, it is very important to conduct specific research on the pathogen (genetic diversity and pathogenic variability, epidemiology and susceptibility to fungicides) in order to develop strategies to control the disease.

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Effect of number of subcultures on in vitro multiplication of four banana clones

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S.C. Patil, A.S. Jadhav,
S.V. Pawar and
B.D. Waghmode

Tissue culture-propagated plants of banana have been extensively planted in India in recent years, mainly to achieve a healthy, early and synchronously maturing crop. However, instances of occurrence of abnormal plants with changed morphology and reduced vigour have been observed in some populations. This could be due to the repeated subculture of the in vitro cultures. Potential hazards of tissue culture have been reported (Danielli 1997). A study was therefore undertaken to investigate the effect of number of subcultures on micropropagation of banana clones.

Materials and methods
Shoot tip explants of four clones: Basrai (AAA), Nendran (AAB), Lal Kela (AAA) and Safed Velchi (AB) were established in vitro, multiplied on MS medium + 6 mg/l BAP + 1 mg/l IBA and rooted on MS media + 3 mg/l NAA, solidified with agar (8 g/l). Five clumps, each with 3 shoots, were transferred to fresh medium in jam jars every 3 weeks for multiplication. Observations were recorded on in vitro multiplication after the 8th, 10th, 12th and 14th subcultures. Plantlets hardened after every subculture were

Table 1. Multiple shoot formation rate of different clones according to subculture cycle.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Average number of multiple shoots/bottle after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8th subculture</td>
</tr>
<tr>
<td>Basrai (AAA)</td>
<td>12.33</td>
</tr>
<tr>
<td>Nendran (AAB)</td>
<td>8.63</td>
</tr>
<tr>
<td>Lal Kela (AAA)</td>
<td>10.72</td>
</tr>
<tr>
<td>Safed Velchi (AB)</td>
<td>8.63</td>
</tr>
</tbody>
</table>

Table 2. Growth response of different clones, using plantlets derived after different numbers of subcultures.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Characters</th>
<th>8th subculture</th>
<th>10th subculture</th>
<th>12th subculture</th>
<th>14th subculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basrai (AAA)</td>
<td>Stem height (cm)</td>
<td>49.09</td>
<td>45.00</td>
<td>31.60</td>
<td>19.70</td>
</tr>
<tr>
<td></td>
<td>Stem girth (cm)</td>
<td>6.50</td>
<td>6.00</td>
<td>4.10</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>No. of leaves</td>
<td>12.00</td>
<td>12.00</td>
<td>9.00</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td>Leaf breadth (cm)</td>
<td>10.50</td>
<td>9.80</td>
<td>6.20</td>
<td>4.90</td>
</tr>
<tr>
<td></td>
<td>Leaf length (cm)</td>
<td>20.50</td>
<td>18.90</td>
<td>15.70</td>
<td>11.60</td>
</tr>
<tr>
<td></td>
<td>Leaf colour</td>
<td>Dark green</td>
<td>Light green, slight leathery leaves</td>
<td>Pale green waxy, leathery leaves</td>
<td>Yellowish waxy, leathery leaves</td>
</tr>
<tr>
<td></td>
<td>Vigour</td>
<td>Normal</td>
<td>Slightly stunted</td>
<td>Medium stunted</td>
<td>Highly stunted</td>
</tr>
<tr>
<td>Nendran (AAB)</td>
<td>Stem height (cm)</td>
<td>55.0</td>
<td>50.3</td>
<td>36.7</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>Stem girth (cm)</td>
<td>7.0</td>
<td>6.2</td>
<td>5.1</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>No. of leaves</td>
<td>10.0</td>
<td>8.0</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Leaf breadth (cm)</td>
<td>7.5</td>
<td>8.2</td>
<td>4.23</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Leaf length (cm)</td>
<td>27.0</td>
<td>23.4</td>
<td>17.4</td>
<td>12.33</td>
</tr>
<tr>
<td></td>
<td>Leaf colour</td>
<td>Dark green</td>
<td>Light green</td>
<td>Pale green, leathery leaves</td>
<td>Highly leathery leaves</td>
</tr>
<tr>
<td></td>
<td>Vigour</td>
<td>Normal</td>
<td>Slightly stunted</td>
<td>Medium stunted</td>
<td>Highly stunted</td>
</tr>
<tr>
<td>Lal Kela (AAA)</td>
<td>Stem height (cm)</td>
<td>59.0</td>
<td>53.0</td>
<td>39.2</td>
<td>29.3</td>
</tr>
<tr>
<td></td>
<td>Stem girth (cm)</td>
<td>5.1</td>
<td>4.3</td>
<td>3.8</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>No. of leaves</td>
<td>8.0</td>
<td>6.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Leaf breadth (cm)</td>
<td>7.8</td>
<td>6.3</td>
<td>5.1</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Leaf length (cm)</td>
<td>18.2</td>
<td>15.2</td>
<td>13.3</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>Leaf colour</td>
<td>Dark green</td>
<td>Pale green</td>
<td>Pale green, leathery leaves</td>
<td>Yellowish waxy, leathery leaves</td>
</tr>
<tr>
<td></td>
<td>Vigour</td>
<td>Normal</td>
<td>Slightly stunted</td>
<td>Medium stunted</td>
<td>Highly stunted</td>
</tr>
<tr>
<td>Safed Velchi (AB)</td>
<td>Stem height (cm)</td>
<td>52.7</td>
<td>52.2</td>
<td>40.3</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>Stem girth (cm)</td>
<td>6.2</td>
<td>6.0</td>
<td>5.0</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>No. of leaves</td>
<td>9.0</td>
<td>8.0</td>
<td>8.0</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Leaf breadth (cm)</td>
<td>6.6</td>
<td>6.5</td>
<td>5.8</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>Leaf length (cm)</td>
<td>20.4</td>
<td>18.2</td>
<td>18.2</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Leaf colour</td>
<td>Dark green</td>
<td>Pale green</td>
<td>Pale green, leathery leaves</td>
<td>Pale green, leathery leaves</td>
</tr>
<tr>
<td></td>
<td>Vigour</td>
<td>Normal</td>
<td>Slightly stunted</td>
<td>slightly stunted</td>
<td>Medium stunted</td>
</tr>
</tbody>
</table>
Table 3. Spectrum of variants clones observed in tissue-cultured plants of different banana.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Variant 1</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basrai</td>
<td>Variant 1</td>
<td>Tall vigorous plantlet, stem pale green, broad long leaves with faint blotching and red margin to peduncle pale green.</td>
</tr>
<tr>
<td></td>
<td>Variant 2</td>
<td>Tall lanky plantlet with long pale green peduncle, stem pale green, long narrow leaves without blotching.</td>
</tr>
<tr>
<td>Nendran</td>
<td>Variant 1</td>
<td>Dwarf, light red stem and peduncle, broad short leaves with red margin and light blotching.</td>
</tr>
<tr>
<td></td>
<td>Variant 2</td>
<td>Dwarf, light red stem and peduncle, narrow long leaves with short peduncle, green leaf margin.</td>
</tr>
<tr>
<td></td>
<td>Variant 3</td>
<td>Dwarf, light red stem and peduncle, narrow long leaves with short peduncle and red leaf margin.</td>
</tr>
<tr>
<td></td>
<td>Variant 4</td>
<td>Tall purplish green stem and peduncle, broad and long leaves with red leaf margin</td>
</tr>
<tr>
<td>Lal Kela</td>
<td>Variant 1</td>
<td>Dwarf, light red stem and peduncle, broad and long leaves with light red leaf margin</td>
</tr>
<tr>
<td>Safed Velchi</td>
<td>NIL</td>
<td>-NIL-</td>
</tr>
</tbody>
</table>

Table 4. Frequency of occurrence of variants in different subcultures of the banana clones.

<table>
<thead>
<tr>
<th>Name of clone</th>
<th>No. of plants and frequency of variants after 8th subculture</th>
<th>10th subculture</th>
<th>12th subculture</th>
<th>14th subculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basrai</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nendran</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lal Kela</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safed Velchi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

observed for their growth in the greenhouse.

Results and discussion

Multiple shoot formation rate varied with the clones (Table 1). Average number of multiple shoots/bottle after the 14th subculture was maximum in Basrai (12.33) followed by Lal Kela (10.72) and minimum in Nendran and Safed Velchi (8.63). Multiple shoot formation rate declined eventually with increase in the number of subcultures in all four clones. The multiple shoots/bottle after the 14th subculture were 7.10 in Basrai, 7.12 in Lal Kela, 6.33 in Safed Velchi and 5.92 in Nendran (Table 1).

The growth of the clones as measured by stem height, girth, number of leaves and leaf size declined after the 8th subculture, with some plants exhibiting very stunted growth after the 14th subculture (Table 2). Safed Velchi was found to be less affected.

Some plantlets which were conspicuously distinct from the parental clones were observed in the populations of hardened plants after the 8th subculture (Table 3). The percentage of variants varied in the different genotypes under study. Gomez and Garcia (1997) also reported similar results. Variations with regards to stature, pigmentation, growth, peduncle and leaf size etc. were seen in the 10th, 12th and 14th subcultures of all the clones except Safed Velchi. Nendran exhibited maximum variants in the 10th (15.87%), 12th (26.58%) and 14th (36.49%) subcultures. Basrai exhibited variant frequency of 1 to 5.55%, while percentage variation was 15.87 to 36.49% in Nendran and 3 to 7.20% in Lal Kela (Table 4).

The percentage of variants in tissue culture propagated plants as high as 91% has been reported previously (Daniells and Smith 1993). Considering the reduced multiplication rate, reduced growth and vigour of the hardened plants and the increased number of somaclonal variations observed after 8th subculture, it may be that for some clones, the number of subcultures in micropropagation should be restricted to eight.

References


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Errata in previous issues of INFOMUSA

INFOMUSA 9(2) – December 2000

Distribution of Blood disease

In the PROMUSA section (p IX), it was reported that blood disease had spread from Indonesia into West PNG. This should in fact have been reported as West Papua (Irian Jaya) and not PNG. Blood disease has not been reported from PNG.

Methodological considerations in the evaluation of banana bunch trimming (Musa AAA, cv. ‘Valery’)

In Vergas and Blanco’s article (p 19), part ‘Material and methods’, 3rd paragraph, it should read: “The following treatments were performed on bunches with eight, nine and ten true hands at flowering: 1) removal of two true hands and 2) removal of three true hands.”

Screening Musa hybrids for resistance to Radopholus similis

In Dohnez et al. (p 3-4), reference is made to TMP 2x 47 and TMP 2x 50. These hybrids should be referred to as TMP 2x 252 S 47 and TMP 2x 252 S 50, respectively.

Consumer acceptability of introduced bananas in Uganda

In Nowakanda et al. (p 22-25), it should read TMP x 551 2 instead of TMP x 551 1 (table 1). The correct identification of the genome of the hybrid TMP x 551 2 is AAAB instead of AABBD (see tables 1, 2, 3 and 5).

INFOMUSA 9(1) – June 2000

In the Musanas news section (p 34-35), the name and contact address of the author of the research on ‘Medicinal weeds in banana orchards in Chhattisgarh, India’ was inadvertently omitted in the English version. It is as follows: P. Oudhia, Department of Agronomy, Indira Gandhi Agricultural University, Raipur-492001, India.
Black Sigatoka outbreak in Australia
An outbreak of black Sigatoka has recently been reported from Queensland, Australia. The area in question is near Tully, in far north Queensland, approximately 140 km south of Cairns. Northern Queensland is the major producer of bananas in Australia and the disease is deemed a significant threat to the region’s $200 million banana industry. There is concern that the outbreak could trigger a very significant increase in the cost of production of bananas in Australia. While far north Queensland has recorded eight outbreaks of black Sigatoka in feral bananas within the past ten years, this is the first time the disease has been detected in a commercial production area. In the past quarantine authorities have used the presence of black Sigatoka in many central American countries as a reason to reject import applications. If this outbreak is not eradicated, it is expected that there will be renewed pressure for banana imports to be allowed into Australia.

Banana breeding in India
Banana breeding was initiated at the Central Banana Research Station, Aduthurai, Tamil Nadu, in 1949. This was one of the first systematic banana improvement efforts in India. The breeding work initiated here has been continued since 1971 at the Tamil Nadu Agricultural University at Coimbatore. The University maintains a collection of 127 distinct accessions and work is focused on hybridisation and selection of offspring with resistance to nematodes, leaf diseases and Fusarium wilt. Screening of the germplasm collection has revealed a number of resistant diploid clones which are being used in the hybridization programme. A number of promising synthetic diploids have been developed and these are being used in further crosses with cultivated diploids and triploids. Some of the newly evolved synthetic hybrids appear to have good levels of resistance to nematodes and leaf spot diseases and acceptable agronomic characters. The conventional breeding programme is complemented by in vitro breeding strategies, which include the creation of variability through mutagenesis and the use of anti-mitotic agents to increase ploidy levels.

Further information about the banana improvement programme of Tamil Nadu Agricultural University are available from K. Soorianathasundaram and N. Kumar, Dept. of Pomology, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India 641003. Email: sooriak@yahoo.com

The banana world loses two friends and colleagues

Ren Gonsalves
INIBAP regrets to have to announce the death of Reynold Gonsalves who died of cancer on February 15, 2001 at the age of 72.
Born in Cuba in 1928, Reynold Gonsalves, of Jamaican nationality, graduated from Howard University, Washington with a Bachelor of Science Degree, majoring in biology.
In 1952 he became Senior Station Master at the Banana Breeding Station, Jamaica and Senior Plant Breeder in 1969. Renn was particularly known in the international Musa community for his research on breeding bananas with resistance to Panama disease and to both yellow and black Sigatoka diseases. He was awarded the order of distinction in the rank of commander for his contribution to agriculture.
Reynold’s work made a considerable contribution to international Musa breeding and he participated in numerous international conferences and meetings. He was a prominent figure in the INIBAP Regional Network for Latin America and the Caribbean and his participation and input was much appreciated.
Reynold Gonsalves was Managing Director of the Jamaican Banana Board since 1996. He was married and has 4 sons and 1 daughter. His other greater interest in life was horseracing and he was Chairman of the Jamaican Racing Commission.

Phil Rowe
On Sunday, 25 March 2001, Dr Phillip R. Rowe died in La Lima, Honduras at the age of 62 years. For over thirty years he dedicated his career to breeding bananas and plantain. He developed a number of important improved varieties that are now being distributed around the world, from Florida to Uganda, where they are bringing substantial relief from the effects of banana pests and diseases, most notably black Sigatoka and Fusarium wilt. Phil was born and schooled in Arkansas. After he graduated from Michigan State University, he moved with his wife, Jeannette, to Honduras to take up a post in what was then the United Fruit Company. He quickly became responsible for the banana breeding programme and has continued to lead the research ever since, seeing through the transition from a private enterprise to a government research institute, the Fundación Hondureña de Investigación y Agropecuaria (FHLA).
The exceptional FHLA hybrids, which Phil developed, are some of the best performing banana varieties in the world. Eight varieties have been made available for trials in the International Musa Testing Programme. They have proved resistant to multiple diseases and pests, highly yielding and consistent in performance over wide ranging environmental conditions. Indeed on the strength of their results, these varieties have been selected for adoption and are gradually being taken up in many banana-producing areas. They will particularly benefit the smallholder producers, farming in marginal areas without pesticides and fertilizers. Where the hybrids have been introduced, they have been taken up with alacrity by farmers. Ongoing projects distributing the varieties to small-scale producers in Nicaragua and Tanzania, have resulted in preliminary increases in yields by a third. However, Cuba, having adopted the FHLA hybrids on the widest scale, provides the most enlightening example. Increased yields without the use of pesticides have had an immediate and impressive impact on farmers’ incomes.
Phil’s conscientious dedication to his work will continue to bring benefits to millions of people worldwide. His generosity, humour and compassion will, no doubt, be remembered by a more intimate circle of friends, colleagues and people who benefited from his kindness. He leaves behind him a wife,
two sons and a grandson. In the following text, his long time colleague and friend, Franklin Rosales, INIBAP regional coordinator for Latin America and the Caribbean, describes some of his memories of Phil and celebrates his life.

In Memoriam
Talking about a friend who has already left for a better place, is difficult to do. One tries to summarize in a few lines all the good things he has done, and quickly finds out that it is not an easy task. Talking about Phil Rowe as a friend, scientist, father, brother, husband, adviser, counsellor, etc., is even harder since he was so good at all of them. Whatever one says about him it will be too little that the intention of highlighting his influence on this earthly life will always be insufficient.

He was a “Good Samaritan”, better than the one mentioned in the Bible, because he cared and loved people not just once but every single day of his life. Every day at the Breeding Station gate at Qumaras, La Lima, or in the dirt road just close to the station, there would be a long line of people waiting to see him to ask for support. Support that they always got without hesitation from Phil’s side. More than one young boy or girl received “scholarships” from Phil for primary or secondary school. Just how many - only Phil and God will ever know since he tried very hard to prevent people from “discovering” the extent of his Charity Ministry. Paraplegics, widows, old timers, sick people were among those on his daily list of protected ones. As his older son Mark says “Something that we will never forget is my Dad’s interest in helping the poor. There was no one that came to our door that left without money, advice or food. He grew up in a very humble family and that’s why he always wanted to help the most needy ones”.

I worked with Phil for more than 10 years and I can testify that these things just touch the surface of Phil’s testimony as a Christian, in the full and true sense of that word. I will never forget that happy smile on his face, no matter the size of the problem we were confronted with and his open hand to give and assist any one asking for help.

He was humble, including when we were reporting to donors. He always said ‘I like to see the donors’ eyes when they come to see me and ask me where the money went or what we are getting from those funds’. When asked about what he wanted for the breeding programme, his answer was quick and always the same: “more pollinators”. He never asked for anything for himself; and never promised more than he could expect or predict from the proposed work. He did not ask even for a new vehicle, when we were riding a very old and beaten up car. Mentioning his material desires, I can tell you that he owned only two cars during his professional life at Honduras (more than 30 years): an old Chevy and a red Toyota car that Jeanette, his wife, had for shopping in San Pedro Sula. The first one, he sold to a Missioner for about $300 and he used to laugh when telling the story. He said “I have never seen a happier face than the one of the Missioner when I told him that the price for the Old Chevy was $300”. He was never worried about money or material things, not because he was a rich fellow but because he had a simple taste for life. He always said: “the ones with the simple taste will survive and live more happily than the rest” He could live happily under a camping tent eating red beans and tortillas. He used to laugh when talking about his experience in the Stock Market where he tried once to bet on Silver. He said, “Franklin, I am so happy that I did not make any money because to tell you the truth I would not know how to spend it anyway”.

Phil was a positive and enthusiastic person, not just in the breeding work that was “his life and love” but also in all other activities. Bad times did not exist for Phil; he always had hope in any situation, no matter how bad it looked to other people. He also had excellent and happy humour with a joke ready in any situation. He used to start his breeding presentations with a joke and he was the first to laugh at it.

He was a very quiet fellow. He withstood stormy times without fighting back, even if those involved were being improper. He was a “Gandhi”, having a “monk’s patience”, trying to solve all problems in a peaceful manner. He “fought” for his crew, to obtain better working conditions and that was reflected every year when the Administration gave him the chance to evaluate his people: They always got the highest marks of all FHIAs staff. He was very proud and dedicated to those working with him, even if could result in a reprimand. He also tried in all possible arenas to convince people that “traditional breeding” was the best alternative for the banana and plantain industry. Few people understood the message or were willing to express views sharing or supporting Phil’s dreams. In fact, very few people appreciate the magnitude of his work and what it will mean for the world in the years to come. Among the few who un-
derstood and appreciated Phil’s work are the Cubans. We travelled together to Cuba and visited all the plots containing FHLA’s hybrids; we went from Havana all the way to Guantanamo. The gratitude expressed by the Cuban people at all levels was the best he ever got and I am pretty sure that he appreciated it and kept it very deep in his heart. As Jose Manuel Alvarez from Cuba expressed in his condolence for Phil “In Cuba we will always remember him with admiration, love and respect; and all those feelings will be materialized in the farms around the Island where today the fruits of his work are flowering”. When we came back to Honduras he kept a wide and happy smile for many days and Jeanette told him “Phil, I do not know what you did in Cuba but I will have to send you back there any time I want you with a happy smile on your face”.

As I mentioned at the beginning, writing about Phil is difficult, because it will never be enough. I would remember him as a very dear friend and boss, as a unique, dedicated and successful banana breeder, but most of all as a person with a great sensibility for the social and human aspects of life. He was humble as all the great scientists are, he was modest, simple, noble, timid and always good. He served the poor in a silent but abundant way. His passion was the development of a better banana or plantain that could be used all over the world to feed the people who depend almost exclusively on this crop. I am pretty sure that Phil’s dream of seeing his banana hybrids all over the world will be fulfilled sooner than later. I just hope that, one day, I can go to same place in heaven where he is now.

Franklin E. Rosales

**INIBAP News**

**New recruitments**

Kim Jacobsen is joining INIBAP as associate scientist in the West and Central Africa office. Her position, financially supported by Vlaamse Vereniging voor Ontwikkelingswerking en Technische Bijstand (VWOB), is specifically focussed on technology development and transfer, and nematology. She has studied at Ghent University in Belgium off and on for seven years in zoology and nematode embryology, completing a Masters thesis and part of the way towards a Ph.D. Taking up her INIBAP position on 1 May, Kim is spending her first three months in Uganda, shadowing Guy Blomme and learning about the Integrated Pest Management (IPM) project taking place in East and Southern Africa. She will then be posted firstly at the International Institute of Tropical Agriculture (IITA) then at the Centre régionale de recherches sur bananiers et plantains CRBP in Cameroon. A large part of her time will be devoted to researching IPM options to limit nematode damage on bananas, carrying out her studies both on-farm and in the laboratory. She will also be dedicating an equal amount of time to developing aspects of technology transfer and assisting in the West and Central Africa office.

Mr Kamulindwa has joined IPGRI-INIBAP as Project Administrator for the Uganda Banana Biotechnology Project. He reported for duty on May 3, 2001. Before joining INIBAP, Mr Kamulindwa worked with the Ministry of Finance of Uganda, CRRE International, CIAT-Africa and Heifer Project International, and comes to INIBAP with a wealthy experience in project management. He will be based at NAROKARI at Kawanda for 75% of his time and 25% at INIBAP regional office in Kampala.

**Asian Agriculture Congress**

A scientific conference, coorganized by the Asian Crop Science Association (ACSA), the Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) and the Federation of Crop Science Societies of the Philippines (FCSSP) on “Food Security and Environment Protection in the New Millennium” took place in Manila, Philippines, on 24-27 April.

INIBAP and IPGRI shared a stall, which they used to illustrate how banana germplasm is distributed worldwide, as well as to display panels and posters with information on INIBAP’s and IPGRI’s networking activities and the importance of bananas and other plant genetic resources to food security. There was also a hands-on demonstration of MUSADOC 2000 and the multimedia CD-ROM on bananas. About 500 agricultural scientists and policy makers from the region and beyond attended.

Other international centres that presented exhibitions included the International Rice Research Institute (IRRI), International Livestock Research Institute (ILRI), and International Service for the Acquisition of Agri-biotech Applications (ISAAA).

**Report on fourth MUSACO Steering Committee meeting**

The fourth Steering Committee meeting of the Musa Research Network for West and Central Africa, MUSACO, took place in Accra, Ghana, on 2-4 April 2001. Ghana’s Minister of the Environment, Science and Technology delivered the opening address. While encouraging the researchers present to continue to develop technologies to augment production of plantain and banana, he lamented the absence of
farmers at the meeting. Professor Walter Alhassan, Director General of the Council for Scientific and industrial research gave the welcoming address and Dr Marcel Nwalozie announced that the West and Central African Council for Agricultural Research and Development (WECARD/CORAF) will provide funds to MUSACO to complete the on-going collection of Musa baseline information in West and Central Africa.

Unlike previous meetings where country reports formed the basis of discussions, this year’s meeting was structured around on-going projects; on periurban banana production, germplasm evaluation and the collection of Musa baseline information, and the presentations of the plantain research team of Ghana. Members were also updated with news from representatives of IITA, INIBAP and WECARD/CORAF.

Scientists from the University of Ghana, Crops Research Institute, the Kwame Nkrumah University of Science and Technology and the Ministry of Food and Agriculture gave brief reports on the various Musa research and developmental activities taking place in Ghana, ranging from nematology and virology to sucker multiplication. For example, at farmer field schools organised by the national integrated pest management project, Ghanaian plantain farmers have been trained to use clean suckers to establish new plots.

Farmers have been selected to take part in the periurban project in Ghana and Benin, and nursery and hardening facilities have been constructed in both countries. In Ghana, the project being implemented by the Crops Research Institute, the Ministry of Food and Agriculture and World Vision Interna-

Views of INIBAP stand at the Asian Agricultural Congress.

Participants of the fourth MUSACO Steering Committee meeting.
ional is taking place around Kumasi and Sekondi-Takoradi, respectively, second and third largest cities in the country. The periurban areas of Cotonou and Abomey Calavi are the sites for the project in Benin, where the implementors are the Institut national de recherche agricole du Bénin (INRAB) and CARDER-Atlantique. Project staff from the two countries have been trained in weaning and hardening of tissue culture plantlets.

The germplasm evaluation trials will be entirely planted out by the end of this year. The weaning and hardening of the tissue culture plants has caused some delay in some of the nine countries involved. The meeting recommended that a training course be organized for scientists and technicians to learn how to handle tissue culture plants. International Institute of Tropical Agriculture (IITA), INIBAP and the network will together look for funds to stage such a course.

Baseline data collection on Musa is taking place in nine out of the 12 member countries but only about four have completed their work. A young professional officer seconded by the Food and Agriculture Organization of the United Nations (FAO) to the MUSACO secretariat will assist in this work and funds will permit the collection of data through surveys.

The coordinator of the International Musa Testing Programme (IMTP) of INIBAP, Dr Jean-Vincent Escalant invited countries to participate in either the ‘in-depth’ or the ‘performance evaluation’ trials. Countries hoping to conduct IMTP trials were asked to nominate candidates for the training course on leaf spot diseases and data collection planned for June 2001 in Asia.

Dr Adiko Amoncho, the West and Central Africa representative on the steering committee of PROMUSA, reported briefly on the PROMUSA meeting that was held in Thailand. He noted the low level of representation of scientists from the region on the working groups. He exhorted country representatives to nominate scientists to one of the five working groups.

A special delegation from the Cameroonian Ministry of scientific and technical research was present to explain the creation of Centre africain de recherche régionale sur bananiers et plantains (CARBAP). The creation of CARBAP demonstrates the willingness of the Cameroonian Government to give a regional dimension to this center which takes over from CRBP.

Participants were informed that as part of the common Musa programme for sub-Saharan Africa, which links activities at IITA and INIBAP, MusAfrica is now being co-published by the two institutions. Members were invited to inform colleagues to send contributions to IITA or INIBAP. Information on MUSACO network activities is posted at the INIBAP and WECARD/CORAF web sites. WECARD/CORAF offered to host electronic discussions on their server.

The President of MUSACO, Mrs Adèle Sambo from Gabon was re-elected and it was decided that the 5th MUSACO meeting would be held in Cotonou, Benin.

**West African scientists visit the Dominican Republic and Costa Rica**

Plantains are an important staple food and cash crop in the lowland humid zones of West and Central Africa. WECARD/CORAF, the sub-regional body coordinating agricultural research and development in West and Central Africa recognised their importance by selecting plantain as one of the priority crops in the sub-region. However, average plantain yields in West and Central Africa are below 10 t/ha, considerably lower than yields in Latin America and the Caribbean, where improved technologies have been adopted.

For 10 days in April 2001, two farmers, four scientists and two extension officers from Benin, Cameroon, Ghana, Côte d'Ivoire and Guinea (Conakry) accompanied by the INIBAP Regional Coordinator for West and Central Africa and the Head of the Department of Seminars and Studies at CTA attended a course on plantain production technologies, which took place first in the Dominican Republic and then in Costa Rica. The Technical Center for Agricultural and Rural Cooperation (CTA) and INIBAP funded the study tour and the Centro para el Desarrollo Agropecuario y Forestal (CEDAF) and the INIBAP regional office in Latin America and the Caribbean provided logistical support.

The specific objectives of the study tour were to:

- Study the different plantain production systems being used in the Dominican Republic and Costa Rica and to compare and contrast these to the situation in West and Central Africa;
- Exchange information on plantain production technologies with Dominican Republic and Costa Rican scientists, extension personnel, and farmers;
- Establish links with plantain researchers in Latin America and the Caribbean through the framework of the INIBAP-coordinated banana net-work for Latin America and the Caribbean, MUSALAC.

Researchers and extension personnel from the Dominican Republic joined in the two days of lectures and discussions led by Dr Sylvio Belalacázar, a Colombian scientist and the brain behind the technologies. The group visited a plantain farmer in Moca, in the Espatat province of the Dominican Republic, who is practising high density planting and harvests 110,000 fingers of plantain per hectare per year instead of the average 27,200 obtained by farmers who rely on traditional practices.

The study group, including scientists from the Dominican Republic, moved to Costa Rica, where they joined with a leader of a plantain marketing cooperative, an extension officer and a researcher from the Corporación Bananera Nacional (CORBANA). Animated discussions took place with several farmers in the Talamanca region of Costa Rica. Bunch yields on these farms have risen from 9-12 kg to 15 to 20 kg since high density planting was adopted and farm incomes have risen sharply as a result.

The basic elements of the technology are:

1. Planting false horn plantains at high densities (2500 to 5000 plants per ha);
2. Using uniform planting materials and
3. Applying fertilizers, fungicides and pesticides at critical developmental stages of the crop.
4. For maximum yield on a piece of land, the crop is re-planted after each harvest.

For the technology to succeed in West and Central Africa farmers should have access to credit to purchase the necessary inputs, which equally must be available at affordable prices. Farmers ought to be able to irrigate areas where rainfall is insufficient in order to support the high evapo-transpirational needs of the high density planting. Marketing is also very important. The participants were unanimous in their desire to see the technology adopted by farmers in West and Central Africa. They agreed to develop a proposal to look for funds to conduct farmer participatory trails to render the technology applicable under the bio-physical and socio-economic conditions of West and Central Africa. Each has also decided to establish his own high density plantation demonstration plot.

**Report of the sixth BARNESA Steering Committee meeting**

The sixth meeting of the Steering Committee of the Banana Research Network

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*INFOMUSA — Vol 10, N° 1*
for Eastern and Southern Africa was held in Zanzibar, Tanzania from 22–23 February, 2001. The meeting was opened by the Hon. Deputy Minister of Agriculture and a welcoming speech was provided by the Hon. Minister of Health. New members to the Committee were welcomed from Sudan and Eritrea.

**BARNESA strategic plan**

The BARNESA Coordinator reported that the evaluation of the ASARECA networks had been completed by the consultants engaged by the European Union. As a result, BARNESA has been classified as an emerging network and will receive limited funding from the EU from July 2001. However continued funding will be dependant on the realignment of the BARNESA strategy towards the 'market-orientated' approach of ASARECA. In order to address this issue, the meeting agreed that a Select Committee should be appointed to work on the finalization of the BARNESA Strategic Plan. Terms of reference and a time frame for the work of the Committee were developed.

The Steering Committee also agreed that the BARNESA priorities as identified in the original strategic plan are still valid but will need to be placed in the context of market oriented research. In this respect, it was noted that marketing experience is lacking in the present Steering Committee, which is composed exclusively of biological scientists. This was recognized as a gap in relation to market-oriented research. It therefore resolved that the Select Committee should be requested to advise on possible new members who could help to fill the recognized gaps in the Committee.

**Ongoing activities**

An update on the banana integrated pest management project (IPM) which is funded by DFID, UK and is being carried out in Kenya, Tanzania and Uganda was provided by Gay Blomme, the Assistant Coordinator for East and Southern Africa. A number of stakeholder meetings have been held at regional and local levels to introduce the project and its aims and objectives. In each country a project site has been selected and baseline data is being collected. IPM options to be tested in the project are being determined in collaboration with participating farmers and all trials will be conducted on-farm. A number of interesting technologies are being considered, including the use of botanicals, ash and cow urine as natural pesticides.

An overview of the banana baseline information project was provided by Charles Eledu, the scientist in charge of this Rockefeller Foundation funded project. Six countries are participating in the project, which is being implemented in collaboration with IITA and NARO, Uganda. It is planned that the project will link closely with the NARO soils programme, which is also funded by Rockefeller Foundation. Equipment for this project, including GIS hardware and software are now in place, and it is hoped that the project will be able to make rapid progress in assembling and analysing available banana research and production information.

Following a presentation on the International Musa Testing Programme by INIBAP's Germplasm Conservation Scientist, BARNESA members were asked to consider participating in the programme by hosting either 'performance' or 'in depth' trial sites. Several countries expressed an interest in becoming part of the ITP.

The BARNESA Chairperson reported on her participation in the PROMUSA meeting in Thailand in November 2000, which she attended as the representative of the NARS of Eastern and Southern Africa. She noted that the prime focus of PROMUSA is genetic improvement and that previously weevils had not been included in the programme. However, during the meeting it was recommended that initial steps be taken towards the formation of a Weevil working group. This is welcome news for the BARNESA region where weevils are one of the main constraints to production.

**INIBAP/IITA joint programme for Africa**

In order to strengthen their collaboration on bananas INIBAP and IITA have agreed to merge their banana research agenda for Africa. This means that the planning and implementation of activities in both West and East Africa will be jointly executed. It is hoped this will improve the delivery of results and facilitate collaboration with NARS. It was noted that the publication Musafrica is now being co-published by the two institutions. Members were invited to inform colleagues to send contributions to IITA or INIBAP.

As the tenure for the Chairperson of BARNESA lasts for two terms, Mary Wabule of KARI, Kenya will continue as Chairperson for 2001-2002. It was agreed that the next meeting could be held in Ethiopia. The Coordinator suggested and it was agreed that the meeting be held in parallel with a national stakeholders meeting to enable committee members to interact with local banana stakeholders.

**Biotechnology project**

Significant advances have been made in the Ugandan government-funded project on the 'Novel approaches to the improvement of the banana production in Eastern Africa – the application of biotechnological methodologies'. The project is bringing together the expertise of the International Institute of Tropical Agriculture (IITA), National Agricultural Research Organization (NARO), Makerere University, Centre de coopération internationale en recherche agronomique pour le développement (Cirad), Katholieke Universiteit Leuven (KUL) and INIBAP to improve the production of East African Highland banana varieties by enhancing their resistance to black Sigatoka, nematodes and weevils. The supervisor of the Tissue Culture Laboratory at NARO, the administrator and four technicians have been selected to work on the project. The laboratory is being equipped and measures are being put in place to ensure that all electrical equipment runs consistently. The 'Coffee building' at NARO research station will also be developed to host a molecular biology laboratory, which will help to enhance on-going studies and build on molecular biology capacities.

A regular supply of male flower buds is being established as starting material for the establishment of embryogenic cell suspensions (ECS). Farmers are currently providing the plant material, but sufficient supplies will soon come from plants grown at NARO research station in Kawanda. Currently, 450 plants representing 4 different cultivars are established. Careful management is planned to ensure adequate control of black Sigatoka, nematodes, weevils and viruses.

A tissue culture training course for project personnel took place on 19-25 April 2001 at NARO, involving three trainers from KUL and Cirad-Département des productions fruitières et horticole (Cirad-Flhor). Methods were taught for inoculating different starting materials, obtaining embryogenic cultures and establishing ECS. More than 200 male buds from 4 different varieties, suckers from 6 different cultivars, as well as scalps from another 6 different banana varieties from the INIBAP Musa Germplasm Collection have been inoculated as a starting point. The supervisor of the Tissue Culture Laboratory, Ms Priver Namanya, will receive further training on the cell suspension methods at KUL and Cirad.

Finally, contacts have been established with potential partners in the United Kingdom, including John Innes Centre, University of Leeds and DFID.
The John Innes Centre have developed and submitted to DFID a complementary project proposal on the genetic transformation of East African Highland Banana. The Cooperation and Development Department at the French Embassy in Kampala also provided a positive reception to a presentation of the project.

**IPM training course**

Within the framework of the DFID funded banana IPM project, a training course was conducted for field technicians and scientists, from 3 till 10 May, 2001. The venue was at NARO, Kawanda Agricultural Research Institute, in Uganda. The training focussed on *Musa* pests and diseases, IPM technologies, cultivar diversity, farmer participatory research methodologies, socio-economics and farming systems.

The course covered broad overviews and a practical training on the use of pest and disease assessment protocols. A farm visit was organised, to pay special attention to both socio-economic aspects of on farm participatory research and to the assessment of pest and disease distribution and incidence.

The course was attended by 15 trainees, comprising of extension officers, NARS research technicians, scientists, NGO representatives, representatives from FAO, Farmer Field School projects, curators of banana collections, a participant from the KCDP project in Kagera, Tanzania and Kim Jacobsen, a VOB associate expert, who is on a three month orientation visit to both INIBAP and IITA programs in Uganda. The acquired knowledge will facilitate the execution of project activities and the dissemination of IPM technologies in the three project countries and beyond.

**MGIS training course and workshop of Names and synonyms in India**

A national *Musa* Germplasm Information System (MGIS) training course was held in India from 21 to 24th of May, at Trichytripally, Tamil Nadu. The course was co-organized with the National Research Centre on Banana (NRCB) under the supervision of Dr Sathiamaorthy and Dr S. Uma. Twelve genebank curators, coming from the major growing banana regions of India: Andhra Pradesh, Karnataka, Kerala, Tamil Nadu, West Bengal and Andaman and Nicobar Islands participated in the course.

The curators were very enthusiastic about MGIS and its value as a tool for the management of germplasm data. MGIS was also considered a very useful medium for the exchange of information on genetic resources with colleagues not only in India but also throughout the region and beyond. This training course brings the number of curators trained in the use of MGIS to 40. MGIS presently contains 4122 records and INIBAP is now working towards making this valuable database freely available for consultation through the Internet.

The MGIS training course was followed by a workshop on Names and synonyms of banana varieties for India. This proved to be an excellent complement to the MGIS training course, providing an opportunity for germplasm curators from all over India to discuss
issues related to Indian banana diversity. A large number of synonyms are recognised for bananas in India, but a number of accessions unique to specific areas were also identified. The important role that MGIS can play in assisting with the clarification of names and synonyms became evident during the two courses.

**Thesis**

Partial resistance assessment of bananas against the black leaf streak disease and evaluation of the aggressiveness variability of the causal agent, *Mycosphaerella fijiensis*


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Banana improvement programmes aim at the creation of new varieties partially resistant to the black leaf streak disease. The principal objective of this study was to characterize the components of this resistance using the infectious life-cycle parameters, to judge their epidemiologic weight and to evaluate the variability of the aggressiveness of the pathogen. Significant differences were detected between partially resistant cultivars for some infectious life-cycle sequences either in natural than controlled conditions. Thus, some resistance components were suggested. The experimental device used in the field did not, however, make it possible to judge the epidemiologic weight of some of them. Elsewhere, a low variability of *M. fijiensis* aggressiveness was detected and no 'cultivar x isolate' specific interaction was highlighted. These results have an implication for the selection of effective and durable partial resistances.

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Dirk’s PhD thesis provides a testimony of his innovative ideas and dedication for improving the *Musa* crop, particularly for African smallholder farmers. As pointed out in the tribute written by his academic mentor Prof. De Langhe, “Particularly striking is the very last paragraph of his thesis with a prophetic character in which his credo is formulated in a few sentences, a credo that is currently proved to be based on a sound and scientifically mature insight.” In Dirk’s words: “A broad-based, improved *Musa* germplasm with pest/disease resistance will be a major component to achieve sustainable production of this vegetatively propagated, perennial crop. Such germplasm can be produced through conventional cross-breeding, enhanced by the utilization of innovative methods for the introduction of additional genetic variation. Also, the increased use of molecular markers will accelerate the process of recurrent selection of improved *Musa* germplasm and, hence, facilitate the development of new hybrids. The prospects of banana and plantain breeding are unlimited and increased efforts will at once initiate a new phase of *Musa* evolution.” This PhD thesis, as well as Dirk’s many other journal articles and book chapters, will be always a source of inspiration to his colleagues and the new generation of scientists involved in germplasm enhancement of research-neglected tropical crops.

**Summary**

Plantain has long been considered intractable in terms of genetic improvement, as only landraces are cultivated despite years of breeding endeavours. Recent advances in several *Musa* breeding programmes have demonstrated that development of improved plantain and banana germplasm through conventional cross-breeding may eventually result in new cultivars for local consumption and commercial production. The recent interest in *Musa* breeding was mainly sparked by the black Sigatoka epidemic, to which resistance is now readily available. Other major production constraints, particularly nematodes, Fusarium and viruses, are now receiving increased attention from breeders. Further progress in breeding may help to make *Musa* a...
modern crop. Seed set rates are workable in many *Musa* subgroups. Insight into combining abilities, heterotic groups, and the genetics of qualitative and quantitative traits has been gained and is being applied to make breeding more efficient. A wide array of breeding schemes is being explored, combining conventional and innovative approaches, and producing potential cultivars from primary tetraploids, secondary triploids and other populations. A number of improved genotypes are undergoing multilocal evaluation, from which knowledge on genotype-by-environment interaction and stability of important traits is acquired. Though some important *Musa* subgroups (Cavendish, False Horn plantain) remain recalcitrant to conventional breeding, biotechnology holds promise for their improvement. However, somaclonal variation through micropropagation has a limited use in plantain improvement as it mostly mimics naturally occurring variation along with the observed poor horticultural performance of somaclonal variants. The purpose of the research reported in this thesis was to foster an increasingly scientific, and not merely empirical, approach to plantain (and banana) improvement. It was also hoped by the author, that his thesis may contribute, be it indirectly, to the much needed transformation of the traditional African agriculture to a modern, science- and technology-based, yet sustainable, system.

**Availability**

A copy of this PhD thesis may be obtained from:
- Prof. R.L. Swennen, K.U. Leuven, Laboratory of Tropical Crop Improvement, Kasteelpark Arenberg 13, B-3001 Leuven, Belgium
  Tel: (32-16) 32 14 20
  Fax: (32-16) 32 19 93
- INIBAP Headquarters, Parc Scientifique Agropolis 2, 34397 Montpellier Cedex 5, France
  Tel: (33) 4 67 61 13 02
  Fax: (33) 4 67 61 03 34

**Musalogue II – Diversity in the genus Musa**

J. Daniels, C. Jenny, D. Karamura and K. Tomkepe
Edited by E. Arnaud and S. Sharrock
INIBAP has just published the second edition of *Musalogue* - a catalogue of *Musa* diversity. This publication provides descriptions and photographs of species and varieties covering most of the diversity in the genus *Musa*. The catalogue is divided into two parts. The first part focuses on the wild species, covering the sections Australimusa, Callimusa, Eumusa and Rhodochlamys, while the second part provides information on cultivated varieties. Each entry in the catalogue is represented by a photograph and a morpho-taxonomic description of the plant in the field. The publication is based on information provided to INIBAP through the *Musa* Germplasm Information System (MGIS) by germplasm curators from Guadeloupe, Cameroon, Australia and Uganda.

Copies of *Musalogue II* are available from INIBAP Headquarters.

**Cryopreservation of Musa germplasm**

Bart Panis and Nguyen Tien Thin
INIBAP Technical Guidelines 5
Edited by J.V. Esclantu and S. Sharrock
ISBN: 2-910810-45-3
This publication describes cryopreservation methods developed for *Musa* tissue at KUL (Katholieke Universiteit Leuven, Belgium) and JIRCA (Japanese International Research Centre for Agricultural Sciences). Detailed protocols are given for all steps to follow from the preparation of the plant material to the recovery and regeneration of whole plants. These methodologies are specific to the type of banana tissue to be cryopreserved: individual meristems, meristem clumps (cauliflower-like structures), embryogenic cell suspensions, and zygotic embryos. Each method is illustrated with figures and colour photographs. Advantages and limitations of each method are highlighted, and current perspectives for optimizing the methodologies presented are discussed. It is hoped that these guidelines will facilitate the adoption and use of the methodologies described. The publication includes bibliographical references and some useful practical information: composition of media and solutions, and list of the basic equipment needed. Available also in French and Spanish.

Copies of this publication are available from INIBAP Headquarters.

**Banana Fusarium wilt management: towards sustainable cultivation**

Edited by A.B. Molina, N.H. Nik Masde and K.W. liew
ISBN: 971-91751-14-1

Fusarium wilt is one of the most devastating banana diseases at the global level and is the number one constraint to banana production in Asia. A first International Conference on Fusarium wilt was held in Miami in 1989, and this resulted in the publication of a book entitled ‘Fusarium wilt of banana’. Since then, many developments have taken place. Research has made significant progress, especially in the development and release of resistant varieties, and in the biochemical and molecular characterization of Fusarium strains. A second International workshop was therefore organized in 1999 in order to
review the current status of Fusarium wilt disease on bananas and identify priorities for future research. The proceedings of this workshop include scientific papers from the banana world's Fusarium wilt specialists covering the themes of pathogen diversity; monitoring and screening methodology; varietal improvement; and disease management. The publication also contains country reports from Asia, the Pacific, Africa and Latin America.

Copies of this publication are available from the INIBAP Regional Office in the Philippines.

A tentative key for identification and classification of Indian bananas

H.P. Singh, S. Uma and S. Sathamooorthy

There is a large diversity of bananas in India, with more than 90 distinct clones having been identified in different genebanks spread across the subcontinent. However, the systematic identification of individual cultivars from one location to another is severely hampered by the large number of synonyms Musa species as well as for the main sub-groups of cultivated bananas. In addition to the classification key, a list of synonyms is provided for each distinct cultivar. The publication includes a large number of colour plates, which illustrate the diversity of bananas in India as well as providing clear descriptions of the taxonomic characters used in the key. This publication is required reading for anyone interested in Musa diversity in India.

Copies of this publication are available from: The Director, National Research centre for Banana (ICAR) #17 Ramalinganagar South extension, Vayalur Road, Tiruchirapalli, 620 017, Tamil Nadu, India. Email: nrcb-sathy@eth.net; nrcbaris@tr.dot.net.in

Organic/environmentally friendly banana production

Edited by F.E. Rosales, S.C. Tripón and J. Cerna


The English version of the proceedings of the workshop "Producción de banana orgánico y o, ambientalmente amigable" held in Costa Rica in July 1998 is now available.

Request for copies can be addressed to INIBAP regional office in Costa Rica.

Cellular biology and biotechnology, including mutation techniques for the creation of new useful banana genotypes

IAEA reproduced in a working document with limited distribution (ref: IAEA-312.D2.RC.579.3) the full version of papers presented during the third FAO/IAEA research coordination meeting on “Cellular biology and biotechnology...” Abstracts of these papers were published in PROMUSA 4 pp. VI-XVI (INFOMUSA vol. 8, No. 2).

Available soon

Two new Musa disease factsheets are presently in press.

The Disease factsheet No. 9 on “False Panama disorder on banana” was prepared by Zaag de Beer, Julio M. Hernández and Sonia Sabadel. The factsheet No. 10 authored by Africano Kangire and Mike Rutherford focuses on “Wilt-like disorder of bananas in Uganda”. Both factsheets will be available in July in English, French and Spanish.
FHIA is seeking a plant breeder

The Honduran Foundation for Agricultural Research (Fundación Hondureña de Investigación Agrícola – FHIA) is seeking an experienced plant breeder to direct and play an active breeding role in its internationally recognized banana and plantain breeding programme located in La Lima, Honduras, Central America. The successful candidate will have an advanced degree in plant breeding, experience in Musa breeding, experience in research administration, and knowledge and experience in modern techniques used in plant breeding. Fluency in English and Spanish languages is desired. A competitive salary, based on qualifications and experience, plus benefits is offered. Interested parties please contact Dr Dale T. Krigsvold, Director of Research at dkrigsvold@fhia.org.hn; Telephone: (504) 668-2809; Fax (504) 668-2313 or send applications with résumés to Recursos Humanos, FHIA, Apartado Postal 2067, San Pedro Sula, Cortés, Honduras 21105 or by E-mail at fhia@fhia.org.hn. Applications will be received until a suitable candidate is found.

Vavilov-Frankel Fellowships 2002

The International Plant Genetic Resources Institute (IPGRI) has established the Vavilov-Frankel Fellowship Fund to commemorate the unique contributions to plant science by Academician Nikolai Ivanovich Vavilov and Sir Otto Frankel.

The Fund aims to encourage the conservation and use of plant genetic resources in developing countries through awarding Fellowships to outstanding young researchers.

The Fellowships will enable the applicants to carry out relevant, innovative research outside their own country for a period of between three months to one year. The research should have a clear benefit to the home country, preferably in areas of the applicant’s future research. Awards can be held concurrently with other sources of support.

In 2002, a total of US$50,000 will be made available for awards. The maximum award per Fellow will be US$25,000 which is intended to cover travel, stipend, bench fees, equipment, conference participation or any other appropriate use. Such research should be linked to innovative topics related to the conservation and use of plant genetic resources such as new conservation technologies and strategies, socio-economic and human aspects of conservation and use, germplasm management, forest genetic resources, policy development, genetic erosion assessment and mitigation and conservation and utilization of specific crops. Work solely on plant breeding or molecular characterization will not be selected. Fellows are encouraged to present the results of their research at an international conference. This can take place within one year of termination of the Fellowship.

Applications for the year 2002 are invited from developing-country nationals, aged 35 or under, holding a masters degree (or equivalent) and/or doctorate in a relevant subject area. Application forms in English, French and Spanish may be obtained from: Vavilov-Frankel Fellowships, IPGRI, Via dei Tre Denari 472/a, 00057 Maccarese (Fiumicino), Rome, Italy; Fax: (39) 0661979661 or e-mail: e.elancy@egiar.org or URL: http://www.ipgri.cia.org/train/avilov.htm and should be returned to IPGRI, Rome. Applications can be sent by mail, fax or email. Applications must be received at IPGRI by 16 November 2001.

Applications must be in English, French or Spanish and should include a covering letter, completed application form, full curriculum vitae, research proposal (maximum 1000 words which should include a clear statement of objectives, feasibility, methodology, materials, justification of the relevance to plant genetic resources, and possible outcomes or impacts), a letter of acceptance from the proposed host institute and a letter of support from the home institute. The successful applicants will be informed by 31 March 2002 and are required to take up their Fellowships before 31 December 2002.

VIth International Symposium of Plant Biotechnology

(1st announcement)
IPB, Cuba, 17-21 June 2002
This symposium is organized by the Institute of Plant Biotechnology (IPB) and Central University “Marta Abreu” of Las Villas, Villa Clara, Cuba.

Topics include: Genetic transformation and Molecular Biology, Tissue culture; Somatic embryogenesis and artificial seed; Massive propagation; Plant improvement through mutagenesis, somaclonal variation and in vitro selection; Decontamination and diagnosis of pathogen microorganisms; Microbial contamination in in vitro tissue culture; Obtaining of secondary metabolites; Information, commerce and intellectual property in plant biotechnology

For further information please contact:
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More details and registration form are also available from: http://www.inibap.org/actualites/villaclara/indexevenin.htm
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Instructions to authors

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

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

- Abstracts: An abstract not exceeding 800-250 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:

- Tables: These should be numbered consecutively and referred to by these numbers 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 originates or is registered in the INIBAP genebank, its accession number (ITC code) should be indicated within the text or in a tabular form.

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


INIBAP/PE/RHID/AC 1996. Descriptors for Banana (Musa spp.).

The following publications are available from Asia and the Pacific office:


A global Programme for Musa Improvement

First meeting of the PROMUSA working groups’ convenors

A first meeting of the convenors of the PROMUSA working groups was held in Montpellier on 18-20 April. Up-to-date news of the activities of each of the five groups were shared and it was agreed that the Genetic improvement working group should continue to operate through two subgroups and not divide into two independent groups as had been proposed. A formulation for two levels of participation in the working groups has been conceived:

- those who are interested in receiving information in order to develop research in general,
- those whose participation is more proactive and involved in the development of priority areas of research in the group.

The convenors will have the responsibility to familiarize themselves with the work of participants and identify those who are most active, and to stimulate information-sharing and the use of the list server. Working group members will be encouraged to send regular updates on publications, meetings, training events and to collaborate in writing project proposals. The PROMUSA secretariat will assist in proposal-writing by making available information on donors, proposal-writing guidelines, background information on banana and plantain production and by assisting in editing and English, if necessary. The responsibilities of INIBAP’s regional coordinators in stimulating participation from all banana-growing regions was emphasized, and the responsibilities of the secretariat in assisting the convenors were also consolidated.

A database of PROMUSA participants will be set up, using and linking to the INIBAP databases, BRIS and MUSALIT. The scope of the database will be relatively broad and participants will be asked to provide information on:

- Materials, tools and methods available for distribution
- Availability of biological materials and conditions to obtain them
- Information about current collaborative activities and novel collaborative areas
- Current training activities, and also areas of expertise and facilities for training.

Changes were suggested for the PROMUSA Web site. Each working group will have its own page containing information on:

- Members (with a link to the proposed database above)
- Research priorities
- Any relevant databases on aspects of research (e.g. Foc database)
- Protocols and methodologies available (with contact details)
- Useful publications: fact sheets, technical guidelines, handouts (Word or PDF versions)
- Links to other relevant homepages

It was also proposed that posters be prepared for scientific meetings, both on PROMUSA in general and on the work of the different working groups. The individual benefits of the global PROMUSA meetings and working group meetings were discussed. Future global meetings should invariably be scheduled back-to-back with another major scientific meeting. The following schedule has been tentatively suggested:

- Nematology working group (24-25 May 2001) after the International Symposium on Nematology meeting in South Africa (21-23 May 2001)
- Sigatoka working group (March 2002) in Latin America back-to-back with an international symposium on banana leaf spot diseases.
2nd International Symposium on the Molecular and Cellular Biology of Banana

The inaugural Symposium on the Molecular and Cellular Biology of Banana held in March 1999 in Ithaca, New York, USA, was organized by the Boyce Thompson Institute for Plant Research. The concept was to open a forum for all people involved in molecular and cellular biology to have an opportunity to meet and exchange information about their research activities. The meeting was a resounding success, and it was therefore suggested to continue the concept under the auspices of PROMUSA.

The 2nd International symposium on the Molecular and Cellular Biology of Banana held 29 October-3 November 2000 in Byron Bay, Australia, was organized by the Queensland University of Technology (QUT) with the local collaboration of CRCTPP (Cooperative Research Center for Tropical Plant Pathology) and QDPI (Queensland Department of Primary Industries). The local organizing committee also received major assistance internationally from INIBAP, Zeneca and DNAP (DNA Plant Technology Corporation, USA). This second symposium allowed participants from both developing and developed countries to present their research activities, covering a broad range of subject areas.

The symposium was structured around the following sessions: genomics; gene expression in transgenic plants; plant pathology and disease resistance; intellectual property and genetically modified organisms; biodiversity and evolution; and biochemistry and fruit ripening. Thanks to the support received from the participating institutions, international scientists were invited to attend and addressed keynotes on “Genomics and banana” (Colin Bird, Zeneca) and “Intellectual property and GMOs” (Dianne Nicoll, University of Tasmania). Participants from the CSIRO (Commonwealth Scientific and Industrial Research Organization) Plant Industry also delivered keynotes introducing the sessions on gene expression in transgenic plants (Peter Waterhouse), plant pathology and disease resistance (Jeff Ellis), and biochemistry and fruit ripening (Simon Robinson).

With 50 papers presented and 60 participants from 17 countries attending the symposium, this event ranks among the most important scientific fora on Musa. As an additional contribution, INIBAP publishes hereunder a special PROMUSA supplement containing the abstracts of presentations made at the symposium.

Abstracts of presentations

Genomics

Induction, detection and use of aneuploids for genetic studies in Musa spp.

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Polyploid and aneuploid banana plants were obtained after gamma radiation and colchicine treatments. Variation in chromosome number was also observed in plants regenerated via organogenesis or somatic embryogenesis from tissue cultures, which were not exposed to any mutagenic treatment. Regenerated off-type plants were analyzed by flow cytometry as described by Dolezel et al. (1997) to estimate their ploidy levels and to check sensitivity of the method to detect aneuploidy in Musa. Chicken red blood cell (CRBC) nuclei were used as internal reference standard and the DNA index was calculated by comparing peak positions of CRBC nuclei and nuclei of the sample. At a triploid level, the minimal difference between euploid (3x) and aneuploid plant (3x ± 1) should be approximately 3%. Thus, all plants with DNA index differing by more than 1.5% from the index established for control (3x) plants were considered aneuploid. The results obtained by flow cytometry were verified by chromosome counting in meristem root-tip cells (Dolezel et al. 1998). The results indicated that flow cytometry was sensitive enough to detect aneuploidy in Musa. However, detection of aneuploidy with ± 1 chromosome accuracy required high-resolution analyses with coefficient of variation of DNA peaks lower than 2%. The advantage of flow cytometric assay was that abnormalities in DNA content could be detected at an early stage of plant growth, and also during in vitro culture. Moreover, flow cytometry enabled detection of mixoploidy. Thus, in several cases differences in ploidy levels between leaf tissue and root tissue of the same plant were detected. Aneuploids have been particularly useful in genetic studies of many plant species such as maize, tomato, tobacco and wheat (Khush 1973). Following the work of Sears, the collection of aneuploid lines has been made possible to define the relationship between chromosomes of hexaploid wheat in terms of their origin and function (Law et al. 1987). In Musa spp., aneuploids are relatively frequent and viable in triploid clones. Being sterile, their value for genetic analyses is limited. Nevertheless, they could be very useful for physical mapping and to link genetic and physical maps using already available molecular markers.

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high resolution chromosome studies in Musa spp. InfoMusa 7:3-4.

Acknowledgements
We thank Ms Ines Van den Houwe (INIBAP) for providing the vegetative clones of Musa and Mr Rony Swennen (K.U. Leuven) for providing Musa embryonic cell suspensions. This work was supported by a Joint FAO/IAEA/GDIFIC (Belgian General Direction for International Cooperation) Coordinated Research Project. The study was undertaken as part of the Global Programme for Musa Improvement (PROMUSA).

Molecular cytogenetic and cytometric analysis of Musa genomes
J. Dolezel, M. Valářík, J. Vránka, M. Dolezelová, J. Safár, M. Lysák, and H. Simková Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc, Czech Republic.
The application of flow cytometry and molecular cytogenetics stimulated progress in understanding of Musa genome at nuclear and chromosomal level. Flow cytometric analysis was found a convenient method for estimation of nuclear DNA content in Musa (Doležel et al. 1994) and has been used for ploidy verification in existing germplasm collections, characterization of newly collected materials, and evaluation of karyological stability in vitro. Due to its high throughput, the method may be easily incorporated into existing breeding programmes. Samples can be sent to laboratories equipped with a flow cytometer, as only a small amount of plant tissue is needed. The method also permits determination of the size of nuclear genome. It was found that Musa genomes are small with the B genome being smaller compared to the A genome (Lysák et al. 1999). The development of procedures for reliable and rapid detection of aneuploidy and for chromosome flow sorting remains a major challenge. Given the small size and poor morphological differentiation of Musa chromosomes (Doležel et al. 1998), molecular cytogenetics holds major promise for karyotype analysis and the study of chromosome organization. While genomic in situ hybridization is suitable for determination of genomic constitution in hybrids (D’Hont et al. 2000), fluorescent in situ hybridization (FISH) permits physical mapping of DNA sequences to chromosomes. Several classes of repetitive DNA sequences, including ribosomal RNA genes, retrotransposon and BSV sequences have already been localized to Musa chromosomes (Balint-Kurti et al. 2000, Doleželová et al. 1998, Harper et al. 1999). More DNA sequences need to be isolated and mapped to unravel the molecular structure of chromosomes and to establish mechanisms of genome differentiation in Musa. Identification of individual chromosomes using physically mapped DNA sequences will allow analysis of their behaviour and segregation during evolution and in breeding programmes. Physically mapped single- and low-copy DNA sequences will provide anchor sites needed to integrate physical and genetic maps.

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Acknowledgements
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Markers for determining genomic integrity: somaclonal variants in bananas as a model system
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Somaclonal variation has long been recognized as a by-product of the propagation of plant cell culture through one or more cycles of disorganized cell growth. Most of the transformation processes used in the generation of transgenic plants include at least one step where cultured cells are grown and then plants are subsequently regenerated. Therefore all of the individuals that are transgenic and have arisen by this method have the potential to contain some of this variation, even in the absence of any visible mutation. Many genomic alterations in transgenic plants have already been demonstrated using RAPDs and AFLPs. In spite of the observation that similar polymorphisms repeatedly arise, none of the variants have proved useful as predictors of the level of genomic variation that has taken place. The well-documented off-types arising from tissue culture of bananas have been used as a model system to identify the regions of the genome that may be especially susceptible to change and to develop markers to determine the extent of that change. Representation difference analysis was used to isolate genomic differences between two sets of normal and variant banana cultivars – between Williams and a masada/chlorotic off-type, and a normal Curare Eno individual and a dwarf off-type (the latter pair supplied by Dr R. Swennen). In both instances difference clones were identified. Many of the sequences were common to both sets of difference products, in spite of the fact that they were different aberrant phenotypes. One of the difference products identified was a minisatellite sequence that also appeared to be labile in date palms. These results add more evidence for the presence of a labile segment of the genome that is preferentially modified during the generation of somaclonal variants. These difference products are being further characterized with a view to developing a series of markers that can be used to identify early genomic changes and also as diagnostics for specific phenotypes arising in the tissue culture process.

Identification of AFLP and ISSR markers associated with dwarf somaclonal variants in Cavendish banana
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Somaclonal variation is a common feature of some micropropagated banana cultivars, caused by undetermined reasons. Early detection of variants is desirable for commercial micropropagation or to establish methods to increase variability for breeding. Molecular markers offer a great potential to detect and to disclose causes of somaclonal variation. The objective of this study was to test Amplified Fragment Length Polymorphism (AFLP) and Inter-Simple Sequence Repeat (ISSR) assays, using polyacrylamide gels and silver staining, comparing a Cavendish cultivar ‘Nanicó Jangada’ with its somaclonal dwarf variant. Twelve ISSR primers were tested, and two (16.6%) presented 3 polymorphic fragment present only in the dwarf variant. All AFLP primer combinations from kit AFLP System I (Life Technologies, Rockville, MD, USA) were tested, amplifying a total of 1665 bands. Each primer combination amplified an average of 26.4 fragments, ranging from 7 to 44 bands. Forty-three polymorphic fragments (2.6%) were identified, with 19 (1.1%) present only in the dwarf variant. Polymorphic fragments were stable between assays. Methylation-sensitive AFLP assay, based on the differential ability of a pair of isochromizers to restrict methylated cytosine, was also tested. A combination of 24 primers were used to amplify DNA from both genotypes. An average of 24.8 fragments were amplified from HpaII-treated DNAs and 22.1 fromMspI-treated, comparable to the regular AFLP. Twelve polymorphic bands (2.1%) were present only in ‘Nanicó Jangada’ in HpaII-digested, while eight fragments (1.6%) were polymorphic for MspI-treated. Only three polymorphisms (0.5%) might have derived from differences in methylation. Other dwarf variants are being tested using the same primer combinations, and polymorphic fragments will be cloned and sequenced.

Application of the amplified fragment length polymorphism (AFLP) and the methylation-sensitive amplified polymorphism (MSAP) techniques for the detection of DNA polymorphisms and changes in DNA methylation in micropropagated bananas

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The effect of the explant source on DNA polymorphisms and changes in methylation in the leaves of micropropagated Musa AAA ‘Grande Naine’ was investigated. Explants were derived from either the young male floral apices or suckers, and shoot cultures induced from these explants were micropropagated for five subcultures. As controls for MSAP analysis (Xiong et al. 1999), equivalent leaf tissue was taken from ten conventionally propagated plants. Ten combinations of primers were used for AFLP analysis and eight primers for the MSAP analysis. No significant differences were found between either kind of explants using AFLP or in MSAP in leaf tissue of plants derived from conventional propagation. However, when compared to the explants, the micropropagated plants derived from them had significantly more DNA polymorphisms. In addition, we found that the explant source had a significant influence on the extent of AFLP DNA polymorphisms in regenerants. Inflorescence-derived regenerants gave the highest variation of 6.36% compared to sucker-derived regenerants which gave 3.96% polymorphisms.

A total of 107 (23%) out of 465 bands were found to be cytosine-methylated in micropropagated plants, whilst in conventionally propagated plants 16% of the bands were found to be cytosine-methylated. There was no significant difference in the extent of DNA methylation polymorphisms between inflorescence-derived micropropagated plants (3%) and sucker-derived plants (1.7%). Most of the polymorphic bands were of high molecular weight (above 700 bp) and were hyper-methylated. This was also the case for most of the hyper-methylated bands common to all micropropagated plants but which were not methylated in conventionally propagated plants. A correlation was found between some plants with AFLP polymorphisms and plants with methylation polymorphisms.

Thus, the banana micropropagation process was found to generate significant genetic and possibly epigenetic changes in micropropagated ‘Grande Naine’ banana plants. The question as to whether the hypermethylation found in all regenerants is developmentally-related or a consequence of the tissue culture environment per se remains to be answered. The correlations found between AFLP and MSAP polymorphisms provide indirect evidence that hyper-methylation may induce base changes, perhaps by deamination (Kaeppeler et al. 2000). All the regenerants are presently being grown to maturity in our experimental plantation in Yucatan so that a phenotypic characterization will be possible.

References

Banana streak badnavirus sequences in Musa
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Molecular and cytogenetic data show unequivocal evidence of the integration of banana streak badnavirus (BSV) sequences into the genome of Musa plantain Obino i’Ewai (AAB) and these sequences are essentially identical to that of an episcopal virus causing infection in Musa (Harper et al. 1999, Ndowora et al. 1999). There are two loci, differing in copy number of BSV sequence in Obino i’Ewai and, for at least one of them, the integrated sequence structure is rearranged with respect to the virus sequence. Significant BSV infections are detected in certain B genome containing Musa germplasm during meiosis or tissue culture and the circumstantial evidence points to episcopal BSV infection arising from the activation or mobilization of integrated BSV sequences. A model involving recombination has been proposed that links integrated sequence to the generation of replicative forms of the virus (Ndowora et al. 1999). This phenomenon has major implications for Musa pathology, improvement, germplasm movement and quarantine.

The BSV integration phenomenon has parallels in other cases, the pararetroviruses Petunia vein-clearing virus (PVCV) (Richert-Pöggeler and Shepherd 1997) and Tobacco vein-clearing virus (TVCV) (Lockhart et al. 2000). Episomal PVCV is found in Petunia hybrida and appears following environmental stress such as nutrient deficiency and episolmal TVCV is found in the hybrid Nicotiana edwardsiorii after changes in daylength. Integrated viral sequences essentially identical to the episomal virus sequences are found at high copy number in both the hybrid species. As is the case for Musa and BSV, the virus sequences are integrated into only one of the parental genomes of the hybrid, although episomal virus is not detectable in that parent. This suggests that the other parental genome plays a part in the “activation” of the virus sequences in the hybrid.

Fragments of a tobacco pararetrovirus-like (TPVL) sequence have been found in genomic DNA of Nicotiana sp. (Jakowitsch et al. 1999). We have shown that pararetrovirus sequences probably comprise an important and widespread component of plant genomes including Gymnosperms and Angiosperms. Their pres-
ence may have consequences for gene silencing and genome evolution. As yet there is no evidence that these sequences give rise to novel viral symptoms, as suggested for related integrated pararetroviral sequences.

We are examining the nature and genomic context of integrated BSV sequences in Obino l’Ewai and in other Musa. A moderately repeated sequence, which flanks the integrated BSV sequence in Obino l’Ewai (MusaOL) is concentrated with varying copy number near the centromeres of most chromosomes of both the A and B genome of Musa. The low numbers of BSV-related integrants per genome indicates that BSV integration occurred after the amplification and distribution of MusaOL sequences and hence is most likely a recent event.

References


Is banana streak virus strain OL the only active virus in the Musa genome?

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In 1999, there were severe outbreaks of banana streak virus (BSV) in plantings of the IRFA 909, 910 and 914 hybrids at separate locations in New South Wales and Queens-

land. These new hybrids, originating from the CIRAD Musa breeding programme, were under evaluation for resistance to Fusarium oxysporum f.sp. cubense for 12-18 months prior to symptom expression. These plants tested negative for BSV-Onne by immunocapture (IC)-PCR. However, IRFA 909 and 910 did test positive for BSV-Goldfinger by IC-PCR. The badnavirus from IRFA 914 was unlike any previously examined. We have named this virus isolate BSV-IM. Using degenerate PCR primers, we amplified DNA of BSV-IM, and used sequence of the DNA fragment, designed virus-specific primers. Using this new PCR assay, we have shown that IRFA 909 and 910 were infected with both BSV-Goldfinger and BSV-IM. In repeated assays over time, IRFA 914 has only ever tested positive for BSV-IM, and not BSV-GF. We have also found BSV-IM infecting an IRFA 914 plant in New Caledonia.

We purified virus from IRFA 910, and have obtained DNA clones representing the whole genome of BSV-IM. We have completed sequencing this virus and initial sequence analyses suggest that the BSV-IM is a distinct virus species. When proteins encoded by ORFs I, II and III of BSV-OL (GenBank Accession AJ002234) and BSV-IM were compared, the sequence identities were 60.5, 42.3 and 64.3%, respectively. We have considered the possibility that BSV-IM has arisen from integrated virus sequences. Our virus clones hybridized to EcoRI and HindIII digested DNA of two diploid B parents of the IRFA hybrid lines, but failed to hybridize to similarly digested DNA from cvs. Obino l’Ewai Calcutta 4 and several AAA cultivars. The virus clones also hybridized to uncut genomic DNA of both diploid B parents. Both diploid B parents have never shown symptoms of BSV infection, and have tested negative for BSV infection by immunosorbent electron microscopy of concentrated leaf extracts. The hybridization patterns observed are not consistent with those expected with episomal virus DNA. These results suggest that BSV-IM has arisen via activation of integrated sequences.

We have also examined the possibility that other strains of BSV are also integrated into the Musa genome. Using probes to the complete genome of BSV-Mys (Geering et al. 2000) we observed complex hybridization patterns with EcoRI and HindIII digested DNA from three diploid B bananas, as well as from cvs. Obino l’Ewai (AAB group), Goldfinger (AAB group) and Pisang Ceylan (MAB group), suggesting that sequence of BSV-Mys is integrated. Likewise, when probed with a 1.3 kb BSV-GF probe (Geering et al. 2000), a ca. 20 kb HindIII fragment was detected in DNA from two diploid B bananas, as well as from cvs. Obino l’Ewai, Goldfinger and Pisang Ceylan, suggesting that sequence of BSV-GF is also integrated. No hybridization was observed between either the BSV-Mys or BSV-GF probes and DNA from a range of AA and AAA cultivars, suggesting that the integrated DNA is linked to B genome of cultivated Musa.

Gene expression in transgenic plants

Agrobacterium-mediated transformation for the generation of transgenic banana (Musa spp.)

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A systematic evaluation of the successive steps in the natural Agrobacterium-plant interaction resulted in the elaboration of an efficient transformation protocol for banana. Chemotaxis and physical attachment of bacterial cells were observed in different cells and tissues of various banana cultivars (Pérez Hernández et al. 1999). Transient reporter gene expression was demonstrated in several tissues cocultivated with vir-induced Agrobacterium and the highest frequencies were found in embryogenic cell suspension cultures. Stable transformation was obtained after selection on geneticin- or Basta-containing medium. In total, more than 600 transgenic plants were regenerated in five independent experiments, and more than 90% of them expressed the induced genes (gfp or gusA). Molecular characterization revealed a simple integration pattern in most transgenic plants. Transgenic plants containing the gene encoding the Ace-AMP1 antimicrobial peptide (Cammue et al.) were screened with a leaf disc bioassay and candidate plants with increased fungus tolerance were identified (Pérez Hernández 2000).

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A novel PCR-based method for the characterization of transgene
insertion in transgenic plants

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An anchored-PCR (APCR) method was developed which allows a fast molecular characteri-
zation of transgenic plants generated via Agrobacterium-mediated transformation. Ge-
nomic DNA fragments obtained by digestion with restriction enzymes are specifically ampli-
fied with a T-DNA-specific primer in combination with an adaptor-specific primer. The incor-
poration of suppression PCR conditions (Siebert et al. 1995) resulted in a significant
improvement and allowed the one-step amplification of specific APCR fragments. Southern
hybridization of T-DNA border-specific probes to the APCR fragments revealed that they were
indeed correctly amplified from the transgene(s). The APCR analysis of a tester set of
20 transgenic banana plants demonstrated that about 70% of them contained one or two
transgene insertions, which compares favorably with the transgene insertion pattern
in plants obtained via microprojectile bombard-
ment (Becker et al. 2000). The technique also allowed the fine structure of the integrated
transgene(s) to be revealed: correct as well as truncated insertions were observed, and
plants containing vector backbone sequences could be identified. In addition, transgenic
plants representing identical transformation events were easily recognized. Finally, nu-
cleotide sequence analysis of cloned APCR
fragments fully confirmed the above findings
(Pérez Hernández 2000).

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Virus and plant-derived promoters for transgene expression
in banana

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Promoter regions derived from banana bunchy
top virus (BBTV) satellite components (S1 and S2) and banana actin genes have been iso-
lated and characterized in transgenic banana
plants. The BBTV S1 and S2 promoters di-
rected vascular-associated reporter gene expres-
sion in both dicots and monocots. In banana,
the activity of these promoters was significantly increased by the inclusion of
monocot-derived introns. Actin gene can-
didates and their associated 3' upstream se-
quen
ces were isolated from a variety of plant
sources, including banana, using a novel ligat-
ion-mediated PCR approach for amplifying
flanking sequences. Expression levels and the
tissue specificity of one particular banana
actin gene (ACT1) were further characterized.
Northern analysis suggested banana ACT1 is
expressed in both reproductive and vegetative
tissues. In transgenic banana plants, the
ACT1 promoter directed strong reporter gene
expression in both leaves and roots. Trunca-
tions of the ACT1 promoter indicated all the
necessary regulatory elements required for
high level (2-fold greater than CaMV 35S)

near constitutive expression are located within
1.2 kb of the ACT1 ATG.

Better bananas - the biotech way

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At DNAP, our efforts in banana are focused on
black Sigatoka resistance, with emphasis at the
early stages of variety development on un-
derstanding expression characteristics of can-
didate gene expression signals. Using the
chimeric uidA gene constructs to assess pro-
moter function, we have been able to identify
several promoters with relatively strong activ-
ity in leaf, fruit and root tissue. These activities
seem to be maintained over several vegeta-
tive generations in the field. Two of these pro-
moters have also been used in experiments to
delay fruit ripening by inhibiting fruit-specific
ethylene synthesis using sense suppression.

Transgenic plants have been assessed in field
trials in Costa Rica and southern Mexico and
several lines have been shown to have signifi-
cant delays in fruit ripening over multiple

gen
erations. A ~23 base RNA fragment diagnostic
of the gene silencing phenomenon has been
identified in these suppressed lines.

Transgenic lines expressing five putative
disease resistance genes are currently under-
going field-testing in Costa Rica. Transfor-

mants expressing 11 more putative disease
resistance genes or combinations of genes
are at various stages of preparation. We are
also using a leaf piece assay to assess some
of our transgenics in house. The symptoms
produced in this assay are similar to those
seen in the field in terms of appearance, tim-
ing and cultivar specificity.

Biotechnological approaches for banana improvement

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Bananas and plantains are the fourth most
important food crop and staple food crop for
millions of people in the developing world.

India is the largest producer of banana in the
world. In this country, banana is the second
most important fruit crop and is grown ~ 0.4
million ha with a production of 10 million tons.
Conventional method of breeding is compli-
cated due to the triploid nature and only a few
diploid clones produce viable pollen. Improve-
ment for disease resistance and productivity
requires the use of biotechnological tools.

Our group is engaged in tissue culture, somatic
embryogenesis, synthetic seeds, in vitro

mutagenesis and selection, DNA fingerprinting
and Agrobacterium-mediated gene transfer.

Thirty cultivars/wild species have been con-
served and propagated in vitro. Tissue cul-

ture-raised plants planted at multilocations ex-
hibited increased yield, early maturity and
more uniform production cycle. In vitro cul-
tures were gamma-irradiated and field evalua-
tion of the irradiated population resulted in
certain promising variants. Isolated variants
and parent cultivars were analyzed in the
field, and at molecular level using RAPDs.

Protocols have been developed for somatic
embryogenesis using shoot tip sections in cv.

Rashnali (AAB) and male flower buds in cv.

Shrimanti (AAA). Embryogenic cell cultures
have been successfully established and maintained by regular subcultures for the past two years (in Rashthali). High frequency conversion of somatic embryos to plants has been achieved and the somatic embryo-derived plants are being field-evaluated.

_Agrobacterium_-mediated transformation using embryogenic cell cultures of cv. Rashthali has been standardized and is now routinely used for gene transfer. Currently, we are working with an anti-microbial peptide, ms199 (a synthetic homologue of Magainin). Studies have shown that this peptide effectively inhibits the growth of _Fusarium oxysporum_, the causal agent of Fusarium wilt. Rashthali, a highly susceptible cultivar, has been transformed with ms199 and transgenic plants have been regenerated.

**Intellectual property and genetically modified organisms**

**Keynote**

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It is probably fair to say that patenting will now usually be in the mind of the genetics researcher for a number of reasons, including:
1. The changing nature of academic science, in particular the need for accountability in economic terms;
2. The nature of biotechnological research: expensive, time-consuming and easily copied; and
3. The increasing involvement of the private sector in the research phase.

The most influential international treaty on intellectual property (IP) rights is the Agreement on Trade Related Aspects of Intellectual Property Rights, or TRIPs, which is an annexure to the WTO Agreement. If a country wants to trade it has to have TRIPs-compatible IP law. Article 27 sets out the following key patent law requirements:

- **27.1**: Patents are mandatory for any inventions in all fields of technology. The elements of novelty, inventive step (non-obviousness) and industrial applicability (utility) must be satisfied.
- **27.2**: Inventions may be excluded to prevent commercial exploitation of the invention to protect public or moral interest, including protecting human, animal or plant life or health and avoiding serious prejudice to the environment.
- **27.3**: Other inventions that may be excluded include: a) diagnostic, therapeutic and surgical methods for the treatment of humans or animals; b) plants and animals, but not microorganisms; c) biological processes for producing plants and animals, but not technical processes. Plant varieties must be protected in one way or another.

(For full text of the Agreement, see WTO Website: http://www.wto.org/english/tratop_e/trips_e/t_agm3c_e.htm)

The patentability of living organisms was uncertain prior to the decision of the US Supreme Court in _Diamond v Chakrabarty_ 447 US 303 (1980). The Court decided that living organisms could be patented by a slim majority (5 to 4). If the decision had gone the other way it may well have led to a decrease in investment in the biotechnology industry.

Case law in the USA and in Europe indicates that the limitations on patentability of biotechnological inventions are not yet fully defined.

1. Courts have interpreted patent legislation to include living organisms.
2. Ordre public/morality arguments are only likely to succeed in the most extreme cases.
3. US Courts are attempting to meet some of the issues associated with broad patent claims.

Article 27 of TRIPs allows member countries some flexibility in deciding which types of biotechnological inventions should be patentable. This, together with the scope for varied interpretation of national IP legislation by national courts, gives individual countries some leeway to provide the level of IP protection that they deem to be acceptable, within the framework of their own cultural, moral and legal norms (trade barriers aside).

Institutions and global programmes, like INIBAP and PROMUSA, have an important role to play in IP management. In particular, they have the capacity to influence decisions about the acquisition of material that may be used to create patentable inventions and the transfer of technology using that material.

**Plant pathology and disease resistance**

The molecular biology of banana bunch top virus

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Banana bunch top disease, caused by _banana bunch top nanovirus_ (BBTV), is considered the most important virus disease affecting banana. The disease is found in almost all banana-growing regions of the world except the Caribbean and the Americas. In the 1920s, bunchy top was the major limiting factor to banana production in Australia. The disease has since been controlled in Australia through the implementation of strict phytosanitary control measures backed up by strict government legislation. Our group has been characterizing this virus for the past 10 years in an effort to develop transgenic virus resistance and to further exploit the virus.

BBTV was initially thought to be caused by a luteovirus based on symptoms, persistent aphid transmission and dsRNA profiles. However, it is now known that BBTV is an isometric virus with a genome comprising at least six different components of circular single-stranded DNA (BBTV DNA-1 to -6) ranging in size from 1018 to 1111 nucleotides. Each DNA component shares a common genome organization including (i) one major gene in the virion sense (except DNA-1 which contains two genes) with an associated polyadenylation signal, (ii) a conserved major common region (CR-M) and stem-loop region (CR-SL), and (iii) a potential TATA box. The CR-M is located 5' of the CR-SL and comprises approximately 92 nt with at least 72% homology amongst the DNA components (except for DNA-1 which has a 26 nt deletion). The CR-M is believed to be involved in replication, where it is thought to act as a binding site for an endogenous ~80 nt DNA primer. The CR-SL comprises 69 nt with at least 62% homology between components. It incorporates a stem-loop structure which contains a 10 bp stem (14 nt conserved) and an 11 nt loop (9 nt conserved). Based on the sequence analysis of DNA-1, -3 and -5, there are two distinct groups of BBTV isolates, the South Pacific group (Australia, Burundi, Egypt, Fiji, India, Tonga and Samoa) and the Asian group (Philippines, Taiwan, Vietnam). These two groups differ by ~10% over the entire nucleotide sequence and by ~30% within the CR-M.

The major gene of DNA-1 contains motifs associated with rolling circle replication and dNTP binding and encodes a replication initiation (Rep) protein. This Rep protein has been shown to possess site-specific nickase and ligase activity (cleaves between nt 7 and 8 of the stem-loop). The function of the internal gene of DNA-1 is currently unknown. DNA-3 encodes the coat protein while the gene product of DNA-5 has been shown to possess reclinoblastoma-binding activity and is thought to be a cell-cycle protein responsible for switching infected cells into S-phase to facilitate virus replication. DNA-4 and -6 appear to encode proteins associated with
cell-to-cell movement and nuclear shuttling, respectively. The function of DNA-2 remains unclear.

BBTV has recently been classified in the genus Nanovirus – viruses with isometric virions which are pleomorphic and possess a multicomponent, circular, single-stranded DNA genome. Other members of this genus include subtropical clan virus (SCSV), faba bean necrotic yellows virus (FBNYV), milk vetch dwarf virus (MDV) and possible coconut foalial decay virus (CDFV).

BBTV DNA-1 to -6 are considered integral to the BBTV genome since these components are consistently associated with all BBTV infections worldwide. Several additional BBTV-associated DNA components have also been isolated from various BBTV infections. Like BBTV DNA-1, these additional components appear to encode Rep proteins. However, they differ from BBTV DNA-1 in several respects, including:

- genome organization – in general, the CR-M and CR-SL are absent, and the TATA box is located 5′ of the stem-loop; and
- they have a limited geographical distribution – they are almost exclusively restricted to the Asian group of BBTV.

We have been examining the replication of BBTV to determine (i) the integral components of the BBTV genome, (ii) which component encodes the “master” Rep, and (iii) the role of the BBTV DNA-1 internal gene. These studies have involved the bombardment of Bgluggoe embryogenic cell suspensions with cloned 1.1mers of the different BBTV DNA components either singly or in combination. DNA was extracted from the cells at 0, 4 and 8 days post-bombardment and analyzed with component-specific probes for replicative intermediates. These studies have shown that DNA-1 encodes the “master” viral Rep protein and represents the minimal replicative unit of BBTV since this component, and not the additional Rep-encoding components, is capable of self-replication as well as directing the replication of the other integral BBTV genomic components. We also showed that the internal gene of DNA-1 is not essential for replication but enhances replication in cis (possibly analogous to the Ren protein of begomoviruses). Finally, we have identified potential Rep-binding sites (Iterons) on the BBTV genome which appear to be similar to those of the begomoviruses. The results of this study have suggested the possibility of two groups of nonviruses: (i) BBTV – which infects monocots and contains an internal gene in the “master” Rep, and (ii) FBNV, MDV and SCSV – all infect dicots and do not possess an internal gene in the “master” Rep.

The epidemiology of banana bunchy top virus in Vietnam

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Banana bunchy top virus (BBTV) causes the most serious virus disease of bananas worldwide. Banana bunchy top disease has been identified in Vietnam from the early 1920s, and similar epidemics have occurred in other countries throughout the world. BBTV was first identified in Vietnam in 1968, and is endemic throughout the country. However, the epidemiology of BBTV in Vietnam appears to be quite different to that observed in other countries, as it does not cause serious epidemics, and appears to move more slowly through the crop. BBTV is transmitted by the aphid Pentalonia nigronervosa, or through infected plant suckers and combs, and typically moves rapidly through a crop. However, in Vietnam it is not unusual to find older BBTV-infected plants adjacent to healthy plants, with banana aphids feeding on all plants. In addition, we have not observed typical BBTV symptoms on the local cultivar Chou tay. It is unknown whether Chou tay is a host for BBTV, or whether it is resistant to BBTV infection. To improve our understanding of BBTV epidemiology in Vietnam, we investigated a number of factors: (1) we investigated the level of sequence variability of DNA-1, the master rep-encoding component, and showed that sequence variability of BBTV in Vietnam is higher than previously recorded in Asia. We also observed that sequences separated into northern and southern Vietnamese isolates, depending on their origin in Vietnam; (2) we identified a new putative satellite DNA component endemic to Vietnam. Finally, we screened Chou tay plants from throughout Vietnam for BBTV, but did not detect virus in any plants using PCR and/or Southern hybridization. This suggests that Chou tay may be resistant to BBTV in Vietnam, which could be one of the factors influencing the epidemiology of banana bunchy top disease in Vietnam.

Viruses and Musa germplasm

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Commercially important Musa spp. include edible bananas and plantains (mostly hybrids of M. acuminata and/or M. balbisiana) and the fibre crop Musa textilis. Six viruses have been characterized from Musa to date (Jones 2000), but additional, uncharacterized viruses have also been recognized.

Banana bunchy top virus (BBTV) has 18-20 nm isometric virions and a multicomponent ssDNA genome. It is transmitted in the persistent manner by the banana aphid Pentalonia nigronervosa and has a scattered distribution in Africa and the Asia-Pacific region. Cucumber mosaic virus (CMV) has 29 nm isometric virions and a tripartite ssRNA genome. It is transmitted in the non-persistent manner by a number of aphid species, and has a widespread international distribution. Banana braact mosaic virus (BBraMV) and Abaca mosaic virus (AbMV) both have filamentous virions, a ssRNA genome and are transmitted in the non-persistent manner by a number of aphid species. AbMV has only been recorded from the Philippines, while BBraMV has a scattered distribution in the Asia-Pacific region. Banana streak virus (BSV) has bacilliform virions (30 x 130 nm) containing a dsDNA genome, and has a worldwide distribution.

The filamentous virions of Banana mild mosaic virus (BanMv) contain a ssRNA genome of 7353 nt, encoding five ORFs. Although related to carlaviruses, foaviruses and potexviruses, the genome organization and phylogenetic relationships of BanMvMv place it apart from all previously described virus taxa (Gambley and Thomas, in press). The virus occurs in a wide range of Musa genotypes and has a worldwide distribution. The virus often occurs as symptomless infections and mixed infections with other viruses, though its mode of transmission is not known. Its economic impact is unknown.

Serological and PCR-based diagnostic assays are available for all the characterized viruses of Musa, but BSV still presents challenges. With BSV, symptoms can be prominent, but occur sporadically. Considerable sequence diversity has been found in BSV, and five of these isolates (BSV-OL, BSV-Mys, BSV-GF, BSV-IM and BSV-Lac) are probably sufficiently distinct to be considered separate viruses (Geering et al. 2000, A.D.W. Geering, N.E. Olszewski, B.E.L. Lockhart and J.E. Thomas, unpublished). Immunoassay (IC) assays are required to differentiate episoal and integrated BSV sequences. IC-PCR with microplate detection has been developed for all the characterized viruses of banana. A multiplex assay for BBmV, BBTV and CMV has been published (Sharmar et al. 2000). Assays for BanMvMv and all known strains of BSV (multiplex) have also been developed (M. Sharmar, A.D.W. Geering, J.N. Parry and J.E. Thomas, unpublished). These assays are
used in conjunction with ELISA and ISEM for routine virus indexing.

All viruses of Musa are transmitted through vegetative propagules, including in vitro plantlets, and this has implications for the health of planting material, the conduct of breeding and transformation programmes and the transfer of germplasm. Virus-free planting material is a major factor in field control of these pathogens and, additionally, several of these viruses have limited distributions. Few studies have been conducted on the transmission of banana viruses through tissue culture. Several studies have shown that, through normal subculturing, a proportion of virus-free meristems arise from initially BBTV-infected clones. This process appears to be accelerated somewhat at elevated temperatures, and plants derived from these meristems remain virus-free (Thomas et al. 1995, and references therein). Recently, the reverse situation has occurred with BSV. Virus infections have been detected in progeny of hybrids from breeding programmes, where there was no evidence of virus infection in the parent lines. This has been shown to be due to ‘activation’ or ‘release’ of BSV sequences that are integrated into the Musa genome (Hull et al. 2000). Recent evidence suggests that several additional strains of BSV may be integrated into different components of the hybrid Musa genome (A.D.W. Geering, N.E. Olszewski, B.E.L. Lockhart and J.E. Thomas, unpublished).

The INIBAP Transit Centre at K.U. Leuven houses the world’s largest in vitro Musa germplasm collection comprising over 1100 accessions. These accessions are being indexed for viruses at three international Virus Indexing Centres (CIRAD, Montpellier, PPRF, Pretoria, and QDPI, Brisbane), and only accessions testing negative for known viruses are released. BanMMV and BSV are the most frequently detected viruses, probably due to frequent latent infection, and the additional factor of BSV integration. BBTV and BBmMV have not been detected in the collection.

References


Elimination of banana and plantain (Musa spp) viral diseases by cryopreservation

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Cryopreservation is becoming a routine in vitro technique which overcomes the serious limitations encountered by the traditional germplasm conservation strategies used in field, seed and in vitro culture collections. The conservation at ultra-low temperatures, usually at –196°C which is the temperature of liquid nitrogen, allows a long-term and contamination-free storage of plant genetic resources. Recently, Brison and collaborators (1997) have demonstrated that cryopreservation could be used, in addition to germplasm conservation, to eliminate viruses from in vitro plum shoots infected with plum pox virus with an eradication rate of up to 50%. The possibility of applying a short (few hours) cryopreservation treatment instead of a long (few weeks) heat treatment would be highly promising.

We previously reported on the successful cryopreservation of proliferating meristems of different accessions of banana, one of the most important staple food crops of the world (Panis et al. 2000). Bananas, which belong to the Musa genus, are found in about 120 countries, mainly tropical and subtropical, on five continents and provide subsistence to millions of people. However, banana plants are threatened by different biotic agents such as bacteria, fungi or viruses, like cucumber mosaic virus (CMV), banana bunchy top virus (BBTV), banana streak virus (BSV), banana bract mosaic virus (BBmMV) and banana mild mosaic virus (BanMMV).

In the framework of an INIBAP project entitled “Development of in vitro culture techniques for the elimination of banana and plantain (Musa spp.) viral diseases”, we aimed to evaluate the effect of cryotherapy on the sanitary state of plant material in comparison with traditional methods such as meristem culture. For this purpose, cryopreservation was performed on meristematic clumps excised from highly proliferating meristem cultures by the vitrification procedure using the PV3-2 solution (Sakai et al. 1990).

Our results show that eradication rates after cryopreservation of highly proliferating meristems reach 39% (32 plants out of 83 tested plants) and 94% (31 plants out of 33 tested plants) for CMV and BSV respectively. In comparison, eradication rates obtained by culture of meristems excised from highly proliferating meristems reached 11% and 63% for CMV and BSV respectively.

Ultrastructural study of highly proliferating meristems performed after a 1-week in vitro culture following cryopreservation showed that cryotherapy acts as a micro-scalpel. Small areas of living cells located in the meristematic dome and at the base of the primordia survive the cryopreservation procedure, while more differentiated cells, distant to the apical dome are killed. Associated with an uneven distribution of viral particles in the meristem, this could explain the efficiency of cryopreservation. The specific localization of viral particles within the meristem is now under investigation. We hope to gain a better understanding of the variations in observed eradication rates according to the virus and according to the therapy.

References


A DNA-based diagnostic test for ‘tropical’ race 4 of Fusarium wilt of banana

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Fusarium wilt of banana is a significant problem to the Australian banana industry. The
fungus which causes the disease, *F. oxysporum* f.sp. *cubense* (*Foc*), is a highly diverse pathogen. At present, only a limited portion of the global diversity of *Foc* has been found in Australia. Thirty-three different vegetative compatibility groups (VCGs) and genotypes of *Foc* have been identified globally, of which nine occur in Australia. Nearly all of the diversity within *Foc* has been identified in Asia, and our proximity to Southeast Asia presents considerable risk of introduction of new strains of *Foc*, and in particular further introductions of the Cavendish-competent strain ‘tropical’ race 4. ‘Tropical’ race 4 is widespread throughout Indonesia and Malaysia, and has recently been detected in Irian Jaya. Several outbreaks of the ‘tropical’ race 4 strain of Fusarium will have already occurred in the Northern Territory, and so far these have been contained by quarantine measures. This strain of Fusarium will pose a threat to the major Cavendish production areas in north Queensland, which are presently free from all Cavendish-competent strains of the pathogen.

We are currently developing a DNA-based diagnostic test that is specific for the ‘tropical’ race 4 strain of *Foc*. We have thoroughly analyzed genetic diversity within *Foc* from the genus to the strain-specific taxon levels using total genomic fingerprinting methods such as DNA Amplification Fingerprinting (DAF) and other PCR-based methods such as restriction fragment length polymorphism (RFLP) and sequence analysis of the ribosomal (r) DNA. We have identified DNA sequence information that is unique to the ‘tropical’ race 4 strain of *Foc* and designed PCR primers that specifically amplify DNA only from the ‘tropical’ race 4 strain. Database searches of DNA sequence information published in Genbank have indicated that there are no matches for these primers with any other organism, but we are currently completing the laboratory screening of the specificity of these primers. We will then adapt our laboratory PCR conditions for amplification of *Foc* DNA directly from infected plants and infected soil. The diagnostic test will then require validation and field-testing, prior to release to industry and/or commercial laboratories.

We are also developing a DNA-based identification system that will allow the accurate characterization of all the strains of *Foc* that occur in Australia. This diagnostic system will allow the detection and identification of *Foc* directly from banana planting material and soil. This system will be useful for screening fields for the presence of *Foc* races prior to planting, screening rhizomes or suckers used for planting material, identifying isolates of *Foc* from infected plant tissue or infested soil, and will also be a useful research tool to study the biology and ecology of *Foc*.

**Isolation of potential disease resistance genes from banana**

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Bananas are susceptible to a wide range of diseases, of which Fusarium wilt and black and yellow Sigatoka are among the most devastating. Although most commercially grown dessert bananas are susceptible to these fungal pathogens, resistance has been identified in wild banana cultivars. A novel approach to identify the resistance genes (R genes) which confer these resistance traits is to amplify genomic DNA using degenerate primers designed to class 3 R genes. This approach has been used successfully on lettuce, soybean, rice and maize but to date no banana R gene candidates (RGCs) have been published.

We have used degenerate primers to amplify five independent RGC sequences from banana, all of which show homology to previously characterized R genes. The five sequences were isolated from both resistant and susceptible cultivars and were present in low copy numbers. In addition, all five sequences were amplified from RNA, indicating that they were transcribed. When the DNA and RNA sequences from resistant and susceptible cultivars were compared, variability was observed between the five RGC sequences (<53% homology) and within each RGC (97-100% homology). Amplification of RGC flanking sequences revealed a single leucine zipper (LZ) domain and a three leucine repeat (LLR) domain, which is consistent with class 3 R genes.

**Banana streak virus promoters are highly active in transgenic banana and other monocot and dicot plants**

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Genetic engineering of plants has proven to be a useful method for the introduction of new desirable traits that are reflected in altered phenotypes, for example enhanced disease resistance. Regulatory sequences or promoters are required to drive efficient expression of the introduced gene in transgenic plants. Viral promoters, such as the 3SS promoter of cauliflower mosaic virus, CaMV (Kay et al. 1987), have been frequently used for constitutive expression of transgenes in many crops. To obtain strong promoters that are suitable for high-level gene expression in transgenic banana, we have analyzed several novel promoter sequences from Australian banana streak badnavirus (BSV) isolates. These were evaluated in different transient and stable transformation assays using reporter genes encoding the green fluorescent protein (GFP) and B-glucuronidase (GUS) reporter enzymes (Schenk et al. 2001). In these experiments, 1322 bp (Cv), 2105 bp (My) and 1297 bp (Go) DNA fragments surrounding the transcription initiation site of the Cavendish, Mysore and Goldfinger BSV isolates (Geering et al. 2000) were analyzed for transcription-promoting activity.

Using transient expression assays, the Cv, My and Go fragments were all shown to have promoter activity in a wide range of plant species including monocots (banana, maize, barley, millet, sorghum), dicots (tobacco, canola, sunflower, *Nicotiana benthamiana*, tipu tree), gymnosperm (*Pinus radiata*) and fern (*Nepirolepis cordifolia*) (table 1).

GUS reporter enzyme activity was analyzed in transgenic *in vitro* grown banana plants (cultivar Three Hand Planty) transformed with the Cv or My promoter constructs. Longitudinal and cross sections of roots, corss, pseudostems and leaves revealed blue staining in all cell types analyzed (for colour photos, visit http://www.uq.edu.au/~ugtreman). The strongest expression was observed in the cortex and the vascular tissue. In roots, a high staining intensity was observed in vascular tissue and emerging side roots. Quantitative GUS activity levels for plants containing the My promoter constructs were higher in leaf, root and cortex tissue compared to plants harboring maize ubiquitin promoter constructs (Table 1). In glasshouse grown banana plants, the My promoter showed higher activities than the maize ubiquitin and cauliflower mosaic virus 3SS promoters (Table 1). The Cv promoter showed activities that were similar to (root and cortex) or higher than (leaf) those of the maize ubiquitin promoter in *in vitro*-grown banana plants, but which were significantly reduced in larger glasshouse-grown plants (Table 1). This may be related to silencing associated with the integrated BSV sequence (Ndowora et al. 1999, Harper et al. 1999) in Three Hand Planty (AAB}
genome) plants. As the integrated BSV sequence is thought to be associated with the 
B-genome, it would be interesting to see if the Cv promoter is more active in AAA-type 
banana plants.

GFP levels in leaves and stems of transgenic sugarcane plants harbouring a Cv pro-
motor/GFP gene fusion were fluorometrically quantified (Remans et al. 1999) and found to 
be comparable to GFP levels in plants harbouring a maize ubiquitin promoter construct 
(Table 1). The expression of both the Cv and maize ubiquitin promoter remained also high 
in ratoon plants of sugarcane. The My promoter was active in young plants but GFP expre-
sion was not observed in mature plants. Strong Go promoter activity was observed in 
transgenic sugarcane callus, but there was no GFP expression in the regenerated shoots. 
The Cv and My promoters were also active in transgenic in vitro-grown tobacco plants, 
but this activity was lost when these plants were grown to adult stage in the 
glasshouse (Table 1).

The promoters from banana streak virus represent useful tools for the high-level expression 
of foreign genes in both monocot and dicot transgenic plants that could be used interchangeably with the CaMV 35S or maize ubiquitin promoters.

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“CIEN BTA-03”, a new somaclonal variant resistant to yellow Sigatoka: 
biochemical, genetic and molecular characterization and agronomic studies 
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In 1996, Trujillo and de García obtained a somaclonal variant resistant to yellow Sigatoka 
by induction of adventitious shoots from the 
triple clone Williams, subgroup Cavendish, 
locally named 'Brasilero', which is susceptible to the 
disease (Trujillo and de García 1996, 
Trujillo et al. 1999). This somaclonal variant is 
not only resistant to the disease, but also 

displays a series of morphological and anatomical 
characteristics that distinguish it from 
triplon clones: a) leaf blade 1.4 times thicker 
than that of clone Williams (Hermoso et al. 
1997, Trujillo et al. 1997); b) lower number of 

cortex per mm² in both upper and lower 
epidermis (Hermoso et al. 1997, Trujillo et al. 
1997); and c) higher phenol content. This 
clone was called CIEN BTA-03 (Figure 1).

The aim of this work is to report the data of the 
biological, biochemical and molecular charac-
terization of CIEN BTA-03, as well as referring 
to the evaluation of the resistant behaviour of the 
variant in the field.

The biochemical studies based on the 
analysis of the proteins by electrophoresis in 
denaturing acrylamide SDS-PAGE gels, 
stained with coomassie blue and scanned in an 
Imaging Densitometer model GS-890 (Bio-Rad) demonstrated the presence of two 
polypeptides (14 and 17 kDa) in the Williams 
clone that are neither observed in the CIEN 
BTA-03 clone, nor in the Fragro 7 (AAAA), 
both resistant to yellow Sigatoka (Giménez 
1998).

Cytogenetic analysis showed that both 
clones presented mosaic tissues, but with a 
different chromosome number distribution; 
22% of the cells of clone Williams have more 
than 33 chromosomes and 78% have less 
than 33 chromosomes. On the contrary, 65% 
of the cells in the resistant somaclonal variant 
CIEN BTA-03 have more than 33 chromo-
somes and 35% have less than 33 (Giménez 

The flow cytometry analysis demonstrated that 
somacline CIEN BTA-03 presents a 
DNA content similar to or higher than that of 
clone Fragro 7 (Figure 2). The values obtained 
in the banana/rice mean ratio (B/R index)

Table 1. Overview of BSV Cv, My and Go promoter activities compared to the CaMV 35S and maize 
ubiquitin promoters in different plant species. Values representing the highest expressing plant: 
GUS enzymatic activity (MU) in n mole MU/h/mg protein and GFP accumulation in mg GFP/mg protein.

<table>
<thead>
<tr>
<th>Transgenic plants</th>
<th>Cv</th>
<th>My</th>
<th>Go</th>
<th>CaMV 35S</th>
<th>Maize ubiquitin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana (leaf in vitro)</td>
<td>1076 MU</td>
<td>8299 MU</td>
<td>nt</td>
<td>nt</td>
<td>214 MU</td>
</tr>
<tr>
<td>Banana (root+callus in vitro)</td>
<td>2502 MU</td>
<td>10650 MU</td>
<td>nt</td>
<td>nt</td>
<td>2571 MU</td>
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<tr>
<td>Banana (leaf+callus)</td>
<td>0 MU</td>
<td>1658 MU</td>
<td>nt</td>
<td>nt</td>
<td>418 MU</td>
</tr>
<tr>
<td>Sugarcane (leaf+callus)</td>
<td>13.1 GFP</td>
<td>&lt; 0.05 GFP</td>
<td>nt</td>
<td>nt</td>
<td>11.6 GFP</td>
</tr>
<tr>
<td>Sugarcane (stem+callus)</td>
<td>5.57 GFP</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>0.80 GFP</td>
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<tr>
<td>Tobacco (leaf in vitro)</td>
<td>0.08 GFP</td>
<td>1.35 GFP</td>
<td>nt</td>
<td>1.68 GFP</td>
<td>nt</td>
</tr>
<tr>
<td>Tobacco (leaf+callus)</td>
<td>&lt; 0.06 GFP</td>
<td>&lt; 0.06 GFP</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>Transient assays</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Maize (sweet corn)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Barley</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Banana</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>Milet</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
<td>nt</td>
<td>+++</td>
</tr>
<tr>
<td>Sorghum</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Carola</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>Tobacco</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
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<tr>
<td>Sunflower</td>
<td>+++</td>
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<td>+++</td>
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<tr>
<td>N. benhamiana</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>Tipu tree</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>Peripterus</td>
<td>+++</td>
<td>+++</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
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<tr>
<td>Faatana</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
</tbody>
</table>

nt = not tested; +++ = strong expression; ++ = moderate to strong expression; + = moderate to weak expression.

INFOMUSA — Vol 10, N° 1
range between 2.92 and 2.99, similar to the tetraploid clone.

Cluster analysis was done using the data obtained by random amplified polymorphic DNA (RAPD) markers for CIEN BTA-03 and 16 different genotypes of Musa spp. (Jiménez 1998, Jiménez et al. 2000, Vidal and de García 2000). Fifty-six polymorphic bands were used for the cluster analyses using Ward’s Unweighted Pair-Group Average (UPGA), and Weighted Pair-Group Average (WPGA) to calculate City-Block (Manhattan) distances. The dendrograms generated by the different methods were identical and showed that CIEN BTA-03 grouped with FHIA-02 (AAAB) and is not closely related to the Cavendish subgroup, to which the parent cultivar Williams belongs (AAA) (Jiménez 1998, Jiménez et al. 2000).

Field evaluation of the resistant character of CIEN BTA-03 (Garcia et al. 2000) shows that this somaclone can be grouped with the cultivar Yangambi km5, based on its resistance to yellow Sigatoka (Figure 3). This somaclone has proved to be also resistant to black Sigatoka (Figure 4).

The efficiency and productivity indexes of CIEN BTA-03 were compared with the FHIA-01, FHIA-02 and FHIA-03 indexes (Garcia et al. 2000). CIEN BTA-03 indexes are very similar in value to FHIA-02 and FHIA-03 indexes (Table 1).

We concluded that we have a new clone resistant to yellow Sigatoka, with a high probability of being also resistant to black Sigatoka, with good agronomic characteristics. It produces a bunch of 34.53 kg and has a productivity index of 0.28 kg per day.

Acknowledgements
This research was supported by a grant under contract G-97000700 from the Consejo Na-

Table 1. Comparison of the efficiency index and productivity index of four banana clones during the second harvest cycle. Experimental Station Samán Mocho, Carabobo, Venezuela.

<table>
<thead>
<tr>
<th>Clone/Cultivar</th>
<th>Genome</th>
<th>Flowering to harvest time days</th>
<th>Bunch weight (kg)</th>
<th>Efficiency index (days/kg)</th>
<th>Productivity index (Kg/days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA-01</td>
<td>AAAB</td>
<td>121.67</td>
<td>26.67</td>
<td>4.61</td>
<td>0.22</td>
</tr>
<tr>
<td>FHIA-02</td>
<td>AAAB</td>
<td>124.77</td>
<td>31.27</td>
<td>3.99</td>
<td>0.25</td>
</tr>
<tr>
<td>FHIA-03</td>
<td>ABB</td>
<td>128.90</td>
<td>36.85</td>
<td>3.47</td>
<td>0.29</td>
</tr>
<tr>
<td>CIEN BTA-03</td>
<td>AAA</td>
<td>121.07</td>
<td>34.53</td>
<td>3.32</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Figure 1. Somaclonal variant CIEN BTA-03.

Figure 2. Flow cytometry analysis of four banana clones.

Figure 3. Evaluation of the incidence of yellow Sigatoka on five banana clones growing in a dry forest at 450 m above sea level. Experimental Station Samán Mocho, Carabobo, Venezuela (1999-2000).

Figure 4. Evaluation of the incidence of black Sigatoka on five banana clones growing in a dry forest at 450 m above sea level. Experimental Station Samán Mocho, Carabobo, Venezuela (1999-2000).
Biodiversity and evolution

Characterization of Musa germplasm held at INIBAP genebank using STMS-PCR markers

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The International Musa Germplasm Collection maintained by INIBAP and hosted by the Catholic University of Leuven (KUL), holds more than 1100 accessions. The objective of

References


Molecular studies on Musa acuminata ssp. malaccensis and selected local Malaysian species

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Current banana is the second largest fruit crop in Peninsular Malaysia and contributes more than RM20 million in export earnings (Jamaluddin 1998).
However, widespread disease problems remain a major constraint to the industry, and require that intensive efforts be made to introduce new resistant cultivars.
The banana programme at the University of Malaya and Universiti Putra Malaysia has recently established a molecular breeding group, which will focus on local indigenous species with a major emphasis on the wild banana Musa acuminata ssp. malaccensis. The programme currently includes an expressed sequence tag (EST) project, STMS analysis, retrotransposon analysis, analysis of potential disease resistance genes and taxonomical studies based on flow cytometry and cytology.

A cDNA library, constructed in a phage vector (tript1ex2), has been established for EST analysis of Musa acuminata ssp. malaccensis genes. Clones from the library are being randomly sequenced and analyzed as part of a long-term banana genomics project. Similarity searches against known sequences deposited in the databases have already revealed identities with genes of known function and with other EST clones. All the sequences obtained will be used to generate a Musa EST database to be used for the further understanding and potential exploitation of banana genes.

Retrotransposon analysis has identified Ty 1-copia-like elements in 10 varieties of banana. A database search showed nucleotide identities ranging between 85-97% and predicted aminoacid identities of between 57-82% when compared to known RT genes of Ty 1-copia-like retrotransposons. The sequences were subdivided into eight distinct groups similar to the Ty 1-copia retrotransposons found in other plant species such as Tto1 in Nicotiana tabacum (Hirochika and Hirochika 1993). Ty 3-gypsy-like retrotransposons have also been isolated with identities ranging from...
55-80% when compared to similar elements in the database. The ubiquity and heterogeneity of the Ty1-copia like and Ty 2-gypsy-like retrotransposons make them a suitable marker for the determination of biodiversity of banana species in Malaysia.

In a separate project, flow cytometry (Dolezel et al. 1991) was used to study ploidy and nuclear genome size variation in Musa species indigenous to Malaysia, i.e. Musa acuminate subspecies, Musa balbisiana, Musa violascens and Musa textilis. No variation was observed in ploidy level, whereas a large amount of variation in the genome size was observed among the different Musa species analyzed. Less variability was observed at the intraspecific level within the species Musa acuminate. Statistical and cluster analysis of data on genome size related in a grouping agreed well with the generally accepted taxonomic classification of Musa.

Studies on disease resistance focus on resistance of local wild bananas to Fusarium oxysporum, the major pathogen of banana in Malaysia. The ultimate aim will be the introduction of resistance genes from the wild species into cultivated varieties using approaches integrating genomics and marker-assisted selection.

The overall integrated approach of the programme, with close links to transformation and breeding groups in the country, hopes to contribute to Musa improvement programmes both locally and globally.

References

Genetic characterization of Brazilian commercial triploid and tetraploid cultivars and wild diploid genotypes using microsatellites
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In Brazil, banana cultivars from subgroups “Pomé” and “Silk” (AAB) are widely cultivated, mostly by small growers. The breeding programme of EMBRAPA Mandioca Fruticultura, Cruz das Almas, Bahia, Brazil, has developed tetraploid hybrids based on a limited number of triploid commercial selections and wild diploids. Identical cultivars with distinct names (synonymous) and distinct genotypes with similar names (homonymous) might be a common phenomenon, and somatic mutations tend to accumulate in banana. The objectives of this work were to characterize 33 triploid commercial cultivars and tetraploid hybrids, plus 49 wild diploid genotypes from EMBRAPA’s breeding programme using microsatellite markers. Primers were purchased from Research Genetics Inc. (Huntsville, AL., USA), and amplified fragments were scored on denaturing polyacrylamide gels stained with silver nitrate. Based on cluster analysis, triploid and tetraploid cultivars grouped according to genome composition (presence of B genome) and to subgroup classification. No difference was detected among cultivars from subgroups “Cavendish” and “Pomé”. Cultivars with erroneous subgroup classification were identified. Tetraploid selections from the same cross were not identical, and presented expected similarity with maternal triploids. Diploids were highly diverse, with the main parental diploid lines employed to develop tetraploid hybrids being very distinct. Some primers amplified more than one locus suggesting that loci duplication might be common in banana, as previously described in the literature. Genetic distances may be used for selecting future crosses.

Studies of Mycosphaerella fijiensis populations structure and of partial resistance of bananas
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The ascomycete fungus Mycosphaerella fijiensis (anamorph Pararoscospora fijiensis) causes black leaf streak disease (BLSD), the most destructive leaf spot disease of bananas (Jones 2000). Knowledge of the extent and distribution of variability within M. fijiensis is necessary for breeding and management of BLSD resistance. A study of the genetic structure of M. fijiensis populations on a global scale showed that individual populations can maintain a high level of genetic diversity and that recombination plays an important role in this pathogen (Carlier et al. 1996). Thus partial but supposed durable resistance should be preferentially used in breeding programmes. The main objectives of this work were to describe the genetic structure of M. fijiensis populations at continental and local scales to evaluate the efficiency and the durability of partial resistance.

To study the population structure of a single pathogenic species, we have first to distinguish this species from close relatives and to determine its distribution. Such a survey, conducted in South and Southeast Asia, led to the discovery of the previously undescribed fungus, Mycosphaerella eumusae (anamorph Septoria eumusae, Carlier et al. 2000). From a taxonomic and phylogenetic study of the ribosomal DNA, we showed that at least nine species, belonging to Mycosphaerella or related anamorph genera, can be isolated from banana leaves (Carlier et al. unpublished). Considering the presence of all these species, the primers defined in the ITS region (Johanson and Jeger 1993) are not strictly specific to either M. fijiensis or M. muscicola. These results show that a good knowledge of the fungal species complex is necessary to develop diagnostic tools. From the phylogenetic study we developed another tool based on a restriction assay of the ITS region and began looking for new specific primers. Such tools should be useful to determine the distribution and the importance of the different species.

The population structure of M. fijiensis at continental and local scales was analyzed from samples collected in Latin America, Caribbean and African countries using eight cleaved amplified polymorphic sequences or CAPS, as molecular markers (Zapater et al. unpublished). Within local populations, we found that most of the genetic variability is distributed on a small scale corresponding to the plant scale. In Latin America and the Caribbean zone, the genetic diversity of M. fijiensis in Honduras and Costa Rica are relatively high compared to the populations elsewhere, suggesting that the pathogen first entered the zone there. Within both Latin America/Caribbean and African areas, a high level of genetic differentiation was detected between most of the populations analyzed indicating that gene flow is limited (Rivas et al. and Carlier et al. unpublished). It is likely that the disease, therefore, has spread in the regions through infected plants and/or through restricted dispersion of ascospores. Continued research at a country level will help to specify the relative importance of both means of transmission. Aggressiveness variability was evaluated in two samples collected in Cameroon and the Philippines by inoculation on five partially resistant cultivars using a leaf
pieces assay (El Hadrami et al., 1998). This variability was similar for both countries although the level of genetic diversity observed in the Philippines is much higher (Carlier et al. 1996). No specific isolate in cultivar interactions were detected. Since only susceptible hosts are cultivated in these countries, results might be explained by the absence of host selection. The potential of pathogen populations to adapt to partial resistance should be analyzed by following their evolution over time in plots of resistant banana genotypes.

To evaluate the efficiency and the durability of partial resistance, three complementary approaches were used: characterization of partial resistance components under controlled conditions, evaluation of the efficiency of these components in the field and population structure analysis of the pathogen. Significant differences between 10 banana genotypes were observed at all stages of the infectious cycle using a leaf pieces assay (El Hadrami 2000). Thus, different components of partial resistance occur at these stages. Epidemiological roles of selected resistance components are currently being studied under field conditions on different plots comprising each a single banana genotype. We are also comparing pathogen population structure between these plots in space and time.

References

New cytological methods to study old problems in Musa L.

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Musa breeding is hampered by a number of constraints including a lack of knowledge of chromosome structure, ploidy and causes of sterility. There are no established karyotypes in Musa because of its poorly staining small, uniform chromosomes and difficulties in obtaining good spreads. Defining correct ploidy levels and establishing techniques for causes of sterility are necessary in Musa breeding. This study describes (i) the use of silver nitrate as a staining agent for Musa chromosomes, (ii) a new procedure to examine meiotic chromosomes in Musa, (iii) ploidy variation in Musa germplasm, and (iv) pollen tube growth in Musa. Acetocarmine, the most common stain used in Musa cytology, is effective for condensed chromosomes such as those in metaphase, but is ineffective for prophase chromosomes. Silver nitrate is shown to be a useful alternative stain for Musa chromosomes. An improved method to examine meiosis in Musa is described. The procedure involves dissection of microsporocytes from the anthers, centrifugation to obtain a large number of microsporocytes, digestion with enzymes and treatment of cells with ethanol-acetic acid. Although Giemsa and Leishman's stain were effective for Musa chromosomes, silver staining was most effective for the less condensed prophase chromosomes. This technique will be useful to develop pachytene karyotypes, characterize new hybrids, and identify nuclear restitution mechanisms (FDR or SDR). Ploidy and genome composition in some of our Musa germplasm showed differences from that of existing data showing the need to better characterize existing germplasm. Finally, a method to observe pollen tube growth in the styles of Musa hybrids will be described.

Biochemistry and fruit ripening

Synthetic seeds in banana: a novel propagation and delivery system
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Edible bananas are vegetatively propagated by suckers as viable seeds are not usually produced. New and effective means of propagating bananas would be advantageous over the conventional use of sucker material, for germplasm maintenance, exchange and also transportation. In vitro culture of vegetative meristems or floral apices is the most promising method for mass propagation. The production of synthetic seeds by encapsulating somatic embryos and vegetative propagules is rapidly becoming an applied technique with potential for mass propagation of elite plant species. Synthetic seed technology will have a significant impact on crop production, in both vegetatively propagated and seed-propagated crops. For the vegetatively propagated plants, synthetic seeds would allow direct planting of clonal varieties and may provide a means for maintenance of elite germplasm.

Synthetic seeds have been prepared by encapsulating shoot tips and somatic embryos and their conversion into plantlets has been studied. Shoot tips of cv. Basrai encapsulated in sodium alginate containing different gel matrices regenerated in vitro on various substrates. Use of Whites medium resulted in high conversion of encapsulated shoot tips into plantlets. Somatic embryos derived from embryogenic cell cultures of cv. Rashthali were also employed for the preparation of synthetic seeds. The encapsulated embryos converted into plants with varying frequencies on different gel matrices and substrates. Plantlets developed from synthetic seeds have been successfully transferred to soil. Synthetic seeds offer a useful tool since they can be handled like seeds and can be useful for storage, delivery and transportation of banana germplasm.

Evaluation of regeneration and transformation systems in Musa acuminate var. Pisang Mas (AA) and Pisang Berangan (AAA)
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Fusarium wilt of banana (Panama disease) is native to peninsular Malaysia and has been recorded as a serious threat to the local industry (Thompson and Johnston 1953). However, attempts at improvement by conventional methods have been impeded by the infertile nature of cultivated bananas. For this reason, we are developing tissue culture and transformation protocols for use on our local banana varieties, Musa acuminate var. Pisang Mas (AA) and Pisang Berangan (AAA) in our laboratory. Regeneration methods have been attempted from single and naked meristems (scaps), meristematic globules and embryogenic calli. Embryogenic calli were derived...
from meristems (Novak et al. 1989) and male inflorescences (Escalant et al.). The highest number of plants regenerated are from scalps. We are now planting these regenerated plants in the field to test for somaclonal variation. We have observed that the regeneration frequency is higher in Pisang Berangan (AAA) than in Pisang Mas (AA). Cell suspensions of both varieties are also being established. Cell suspensions from male inflorescences developed at a faster rate than those from apical meristems.

Plant transformation using both biolistic and Agrobacterium methods were attempted. Scalps and embryogenic calli were shown to be most responsive in the transformation experiments. Histochemical assays were used to optimize transformation parameters and identify suitable explants. Cell suspensions of both varieties will be used for transformation in future.

We are also isolating the antifungal gene from wild banana, Musa acuminata ssp. malaccensis. According to published data, this species is known to be resistant to Fusarium wilt races 1 and 4 (Vakili 1965).

Innovations for the commercial production of tissue culture plants are also being developed. We have developed a chamber which we call a «steriponics chamber» which merges the principles of tissue culture and aeroponics. The advantages of this chamber include faster plant production, minimum contamination risk and less dependency on labour. This chamber could also be used for physiological and pathogen assessment experiments.

A data tracking system has also been developed to monitor plant production using a bar coding system. The use of this system would allow monitoring of virus-indexed plants and quality control, and provide the necessary production data.

References