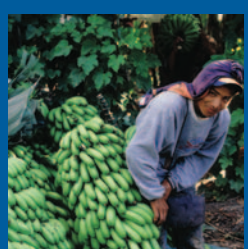
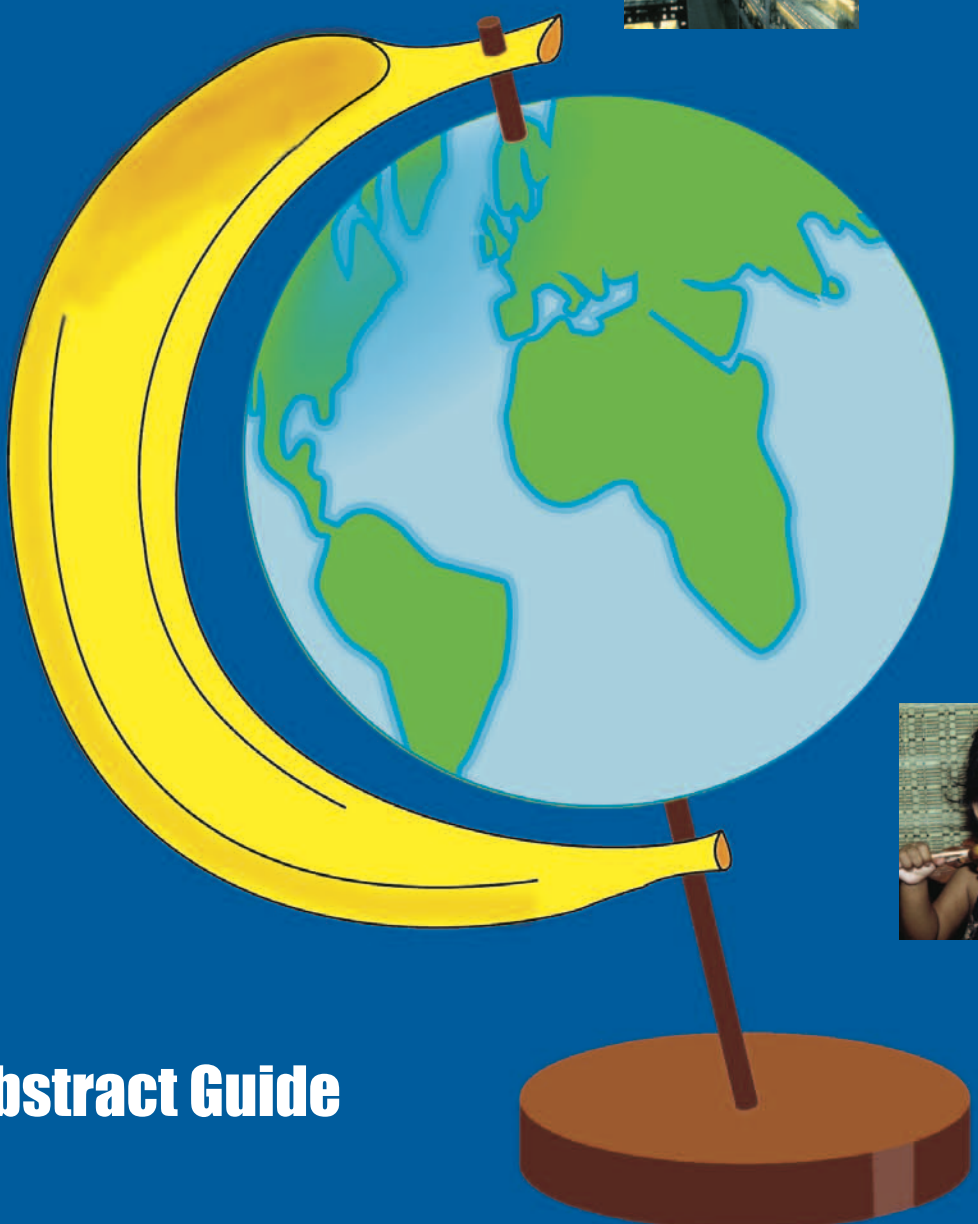
1st International Congress on *Musa*

Harnessing research to improve livelihoods

6-9 July 2004, Penang, Malaysia





Abstract Guide

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IPGRI

The mission of the **International Network for the Improvement of Banana and Plantain** (**INIBAP)** is to increase the productivity and stability of banana and plantain grown on smallholdings for domestic consumption and for local and export markets.

INIBAP has four specific objectives:

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

INIBAP is a programme of the International Plant Genetic Resources Institute (IPGRI), a Future Harvest center.

The International Plant Genetic Resources Institute (IPGRI) is an independent international scientific organization that seeks to advance the conservation and use of plant genetic diversity for the well being of present and future generations. It is one of 15 Future Harvest Centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. IPGRI has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme and (3) the International Network for the Improvement of Banana and Plantain (INIBAP).

The international status of IPGRI is conferred under an Establishment Agreement which, by January 2003, had been signed by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mauritania, Morocco, Norway, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovakia, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine.

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Session 1

Genetic resources and improvement

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Keynote lecture: Conventional breeding strategies for *Musa* improvement and their world status

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Banana and plantain production is severely threatened by several pests and diseases. Furthermore, wind damage severely affects yield. Apart from the Cavendish bananas produced for exportation by multinational companies, the other dessert bananas and most plantains and cooking bananas are mostly cultivated by small-scale farmers for home consumption and for sale in local and regional markets. Since these farmers cannot afford pesticides, resistant varieties appear as an appropriate and environmentally friendly solution for sustainably improving yield.

Cultivated bananas are mostly triploid and parthenocarpic. Being highly sterile, they are difficult to breed and little knowledge on *Musa* genetics and cytogenetics is available. Despite these constraints, progress has been made in conventional genetic improvement of *Musa* in recent years and some new varieties derived from popular regional cultivars are now becoming available from major conventional breeding programmes.

Based on the large number of accessions collected in the primary and secondary zones of banana diversity, the initial breeding approach was focused on triploid x diploid crosses (3x/2x) aimed at producing disease-resistant tetraploid hybrids from partially fertile triploid cultivars. Using resistant diploid clones as male parents and exploiting non-reduced 3x female gametes produced by partially fertile female triploid cultivars, has produced, through embryo rescue, the production of several disease-resistant tetraploid hybrids bearing traits from the mother parent. Improved diploid parents from extensive 2x/2x crosses have also been used to optimize the production of superior hybrids.

Moreover, this 3x/2x approach led to the creation of diploid hybrids that are used to develop elite diploids, among other breeding strategies. Previous efforts, namely at the former Jamaican banana breeding programme and later at FHIA, have produced improved dessert-type diploid parents with interesting resistances and agronomic performance. More recently, cooking and plantain-type diploids have been developed and are now available from CARBAP, IITA and EMBRAPA. Based on these improved diploids, FHIA, CARBAP, EMBRAPA and IITA are focusing their strategies on developing triploids by combining them with primary or secondary tetraploids.

In vitro tissue culture and mutagenesis have allowed the emergence of another 4x/2x strategy, aiming at re-creating triploid hybrids from ancestral diploid material. Extensively used by CIRAD to develop triploid dessert banana, this strategy relies on a very good knowledge of banana genetic resources and of phylogenetic links between ancestral and present varieties. The induced tetraploid parent is obtained through colchicine treatment of a diploid one. Using a higher range of fertile genitors, this strategy has led to the creation of large hybrid populations of AAA and AAB triploids. This strategy could benefit from widening of the genetic base of the crosses and from marker-assisted selection. Both these strategies are however limited by the problem of BSV activable sequences in the B genome, which prevents their use in breeding.

Some major breeding programmes are targeting the creation of high-yielding small stature or dwarf/early hybrids and the first interesting products are already produced. This overview indicates that much is possible with conventional breeding, although not everything. Conventional breeding is just one part of an integrated improvement banana scheme. Recent developments in breeding strategies and new hybrids from popular varieties will be presented, and approaches to reinforce and enhance the promising results of conventional breeding will be discussed.

State of the art on the use of molecular markers for diversity studies

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Abstract not available

Morphological classification of *Musa acuminata* Colla in lower northern Thailand

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This research was conducted in seven sites scattered over lower northern Thailand during 2001-2003. Three hundred and thirty six samples were collected along line transects and classified into 7 groups. Group 1 was *Musa acuminata* ssp. *malaccensis* (Ridl.) Simmonds, which shared some characters with ssp. *siamea* Simmonds and ssp. *burmannica* Simmonds. Group 2 was called “Mae Wong Form”, because of dominant characters such as the prominent 1/3 bright light green or viridescent on the bract’s external face and strongly convoluted bract tips. This group was different and relatively isolated from ssp. *malaccensis* (Ridl.) Simmonds. Group 3 had the prototype characters of ssp. *malaccensis* (Ridl.) Simmonds. Group 4 showed all major characters of ssp. *malaccensis* (Ridl.) Simmonds, but also shared some characters with ssp. *banksii* (F.v. Muell.) Simmonds. There were new characters of glossy pink purple on the bract external face and bright orange red on the bract’s internal face. Group 5 was ssp. *siamea* Simmonds. However, variations in male bud were observed in three populations; 28.9% of male buds were different from the prototype. Group 6 was identified separately away from *M. acuminata* Colla due to the prominent erect rachis position. Finally, Group 7 was the typical ssp. *burmannica* Simmonds. There were more variations of *M. acuminata* Colla than previously recorded. All groups could be promising genetic materials for breeding due to their vigorous growth and agronomic characters.

Endemic Callimusa species of Borneo

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In prehistoric times, Borneo was connected to the Asian mainland. To the east of the island is Wallace's line, the dividing line between the Oriental and the Australian flora and fauna. Because of the plant species that developed as a result of its isolation from the continent, Borneo is considered as a center of plant, and also banana, biodiversity in tropical Asia.

Borneo harbours a large number of wild banana species. As banana plants prefer open areas, they are usually found in small and isolated populations. They consequently manifest genetic variation. Until the end of the 19th century, the island was covered with dense rainforests but agriculture and logging, in particular, have led to the clearing of the forest and as such the expansion of wild banana growing areas.

The following endemic species belonging to the Callimusa section, have been observed in Borneo and described by Häkkinen: *Musa bauensis, Musa beccarii*, *Musa borneensis*, *Musa campestris*, *Musa flavida, Musa hirta, Musa lawitiensis, Musa monticola, Musa muluensis, Musa suratii, Musa tuberculata* and *Musa vooni.* In addition, *Musa pigmaea* was rediscovered in Sabah but has not yet been described.

These populations are relatively homogenous and hybridization has not been observed. All these species/varieties are eventually expected to hybridize into one homogenous population. *Musa* seeds can remain viable for decades and will germinate rapidly when an opening in the canopy is created. Small mammals, such as squirrels and monkeys, contribute to the dispersal of the seeds.

Germplasm is currently stored at the Sarawak Agricultural Research Centre and the Universiti Malaysia Sabah. Given that these Callimusa species are resistant to many diseases and as such could contribute to the breeding of resistance, genetic studies should be initiated

New *Musa* species can still be found. With the discovery of *Musa voonii*, the number of native *Musa* species in Borneo has increased to 14.

Genetic diversity of cultivars from southern India using RAPD markers

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Southern India is a major center of diversity of cultivated bananas. The numerous cultivars and mutants representing the AA, AAA, AB, AAB and ABB groups display striking variation in the morphology of the plant, bunch and fruit. Much of the early work on characterization and classification of *Musa* cultivars and clones was based on morphological characters. Molecular techniques have emerged as useful tools in understanding the genetic relationships between banana cultivars and species. The random amplified polymorphic DNA (RAPD) technique[1] is being widely used to better characterize individual accessions, identify duplicates and track clonal variants[2, 3]. At the Banana Research Station in Kannara, 256 accessions are conserved in the field genebank. Morphological characterization of the accessions has facilitated the identification of distinct cultivars/clones and their synonyms.

In this part of the study, genetic diversity in 35 morphologically distinct indigenous banana cultivars representing five genomic groups (listed below) and available in the genebank in Kannara was assessed employing RAPD analysis.

AA	AB	AAA	AAB	ABB
Matti	Kunnan	Amritsagar	Sugandhi	Kanchikela
Namrai	Adukkan	Robusta	Thulsimalbhog	Chetti
Kadali	Adakka kunnan	Chakkarakeli	Nendrakali	Monthan
Sannachenkadali	Padalimoongil	Chenkadali	Krishnavazha	Sambranimonthan
Annaikomban	Kodappanilla kunnan	Pachakappa	Virupakshi	Sakkai
Calcutta 4	Njalipoovan	Manoranjitham	Kullan	
			Nendrapadathi	
			Thiruvanantha puram	
			Chinali	
			Mysore Ethan	
			Dudhsagar	
			Velipadathi	

DNA was isolated from tender banana leaves using liquid nitrogen and CTAB extraction buffer. Forty random decamer primers (OPF, OPE, OPU, OPAK and OPAH series) were used to amplify the DNA. PCR was carried out in 45 amplification cycles and the products separated by electrophoresis on 1.5% agarose gel, containing 0.5 µg/ml of ethidium bromide. The RAPD bands were visualized under UV light and photographed. Coefficients of similarity among cultivars were calculated. RAPD markers were used to elucidate the level of variation between cultivar groups. A dendrogram of the cultivars was constructed using the Ntsyspc 2.0 programme. The cultivars were grouped in clusters that generally follow the taxonomy based on morphological characters.

1. Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

2. Jarret R.L, D.R. Vuylsteke, N.J. Gawel, P.B. Pimental and L.J. Dunbar. 1993. Detecting genetic diversity in diploid banana using PCR and primers from a highly repetitive DNA sequence. Euphytica 62(1-2):69-76.

3. Howell E., H.J. Newbury, R.L. Swennen, L.A. Lithers and B.W. Ford Lloyd. 1994. The use of RAPD for identifying and classifying *Musa* germplasm. Genome 37:328-332.

Improvement of East African highland bananas

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Banana (*Musa* spp.) is an important staple and income-earning crop for millions of people in the highlands of East Africa. The crop is affected by a number of production constraints including black leaf streak disease, banana weevils, nematodes, Fusarium and bacterial wilt, and viruses. In response to the declining yields of the crop in East Africa, IITA set up a banana breeding programme in 1995 in Namulonge, Uganda. The emphasis was to determine male and female fertility in the East African landraces and set up a conventional breeding programme for the genetic improvement of bananas. The project aimed at developing resistance to black leaf streak disease and other pests, germplasm enhancement using biotechnology and development of sustainable production systems. The initial breeding approach was the production of tetraploid progenies from $3x \times 2x$ crosses.

Flow cytometry is used routinely to determine the ploidy of the progeny. Diploids are also selected and are useful for establishing diploid $\times$ diploid populations and as male parents. We have selected a number of tetraploids with increased black leaf streak resistance and good bunch characteristics. These tetraploids were then crossed with improved diploids to produce secondary triploids. We have selected 16 secondary triploids with various levels of black leaf streak resistance and good bunch characteristics. Five of these hybrids have been selected for evaluation in farmers' fields. On the basis of this evaluation the hybrids will be made available for wider distribution.

Improved hybrids from Brazil

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Brazil is the third most important producer of bananas in the world, with six million metric tones produced on 600 000 hectares. Sigatoka disease, black leaf streak disease and Fusarium wilt are the most serious banana diseases in Brazil, causing yield losses that may reach up to 100%. Fusarium wilt is the main limiting factor for the cultivation of 'Silk' (Maçã) and is also a problem for the popular Pome cultivars such as 'Prata', 'Prata Anã' and 'Pacovan'.

Two hybrids developed at Embrapa have been recommended for planting: the tetraploid (AAAB) hybrids 'Tropical' and 'Preciosa'. 'Tropical' was obtained by crossing 'Yangambi 2' with the diploid M53 (AA), which presents plant growth characteristics and yields similar to those of 'Maçã'. 'Tropical' is also resistant to Sigatoka disease and tolerant to Fusarium wilt. Since it has about the same height as 'Maçã', it can be grown using the same spacing and will give similar yields in Fusarium wilt free areas, i.e. 10 to 20 t/ha when grown under rain-fed conditions and up to 30 t/ha under irrigation. The hybrid has a good budding capacity and requires relatively deep soils for growth and development. Ripe fruits have a yellow rind and a whitish sweet pulp that has a low acidity and a taste similar to the one of 'Maçã'. Other fruit characteristics, such as total soluble solids, total tritable acidity and ratio are all similar to those of 'Maçã', which should facilitate its acceptance by consumers.

'Preciosa' is a Prata type hybrid that was obtained by crossing 'Pacovan' (AAB) and the diploid M53. Its growth and yield characteristics are similar to those of 'Pacovan'. 'Preciosa' is resistant to black leaf streak disease, Sigatoka disease and to Fusarium wilt. It has shown outstanding agronomic and fruit qualities in several ecosystems, compared with 'Pacovan'. The plant is tall, presents good budding and requires deep soils for growth and development. Ripe fruits have a yellow rind and a yellowish white sweet pulp with low acidity. Besides being as rustic as 'Pacovan', 'Preciosa' grown under irrigated conditions and with an adequate supply of fertilizers can yield 35 to 40 t/ha. Total soluble solids, total tritable acidity and ratio are similar to those of 'Pacovan', thereby increasing the chances of consumer acceptance.

Banana breeding at Tamil Nadu Agricultural University

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Tamil Nadu in India is endowed with a rich variety of bananas and plantains. The commercially important cultivars are 'Robusta' (Cavendish, AAA), 'Red banana' (AAA), 'Rasthali', also known as 'Silk' (AAB), 'Poovan', also known as 'Mysore' (AAB), 'Virupakshi', also known as 'Lady finger' (Pome, AAB), 'Nendran', also known as 'French plantain' (AAB), 'Karpooravalli', also known as 'Pisang awak' (ABB), 'Monthan', also known as 'Bluggoe' (ABB) and 'Ney poovan' (AB). The prevalent diseases and pests include Sigatoka disease, Fusarium wilt, nematodes, rhizome and stem weevils, bunchy top disease, bract mosaic disease, banana mosaic disease and banana streak disease.

Improvement of bananas at Tamil Nadu Agricultural University incorporates both conventional and innovative breeding techniques. Many synthetic diploids resistant to Sigatoka disease and *Radopholus similis* have been developed[1]. Diploid x diploid crossings and triploid x diploid crossings have resulted in the development of many synthetic diploids, triploids and tetraploids. Evaluation of these hybrids for yield, fruit quality and resistance to nematodes, Fusarium wilt and leaf spot disease[2,3] has led to the identification of many potential hybrids. The breeding potential of these hybrids is discussed.

In vitro mutation breeding by using gamma irradiation and chemical mutagens (ethyl methane sulfonate) has resulted in the creation of superior mutants of 'Robusta' with good bunch traits (bunches weighing over 28 kg). Evaluation of mutants of 'Poovan', 'Red banana' and 'Nendran' is now in progress. The i*n vitro* induction of polyploidy in diploids, such as 'Sannachenkadali' (AA), 'Anaikomban' (AA), 'Kunnan' (AB) and 'Thattillakunnan' (AB), has resulted in the synthesis of 24 tetraploids that are being evaluated for their breeding potential. Reversion to diploidy was observed in many cases during field evaluation and a few of the diploids turned out to be triploids when subjected to flow cytometry in the third generation. The significance of the variants developed through these innovative breeding techniques will be discussed.

1. Sathiamoorthy S. 1987. Studies on male breeding potential of some banana clones. M.Sc. thesis, Tamil Nadu Agricultural University, Coimbatore, India.

2. Kishnamoorthy V. 2002. Breeding for resistance to sigatoka leaf spot and nematodes in banana (*Musa* spp.). Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, India.

3. Damodaran T. 2003. Breeding for resistance to fusarium wilt and nematodes in banana (*Musa* spp.) Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Evaluation of improved hybrids in Kerala, India

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Banana is the most consumed fruit in Kerala, India. Located within the center of diversity of cultivated bananas, this southern State accounts for a major share of variability in banana and plantain cultivars, particularly the AAB, ABB and AB groups. Bananas are grown in a wide range of environments with other crops or as a monocrop and provide a steady source of income to growers. In recent years, pests and diseases have increasingly threatened a number of prominent traditional cultivars.

The Banana Research Station in Kannara is engaged in the collection, conservation and evaluation of banana germplasm and serves as a nodal center distributing clean planting material to the farmers of Kerala. In 2001, sixteen accessions, including nine improved hybrids: SH-3640, SH-3436-6, TMBx5295-1, TMBx1378, FHIA-17, FHIA-18, FHIA-25, FHIA-21 and CRPB-39, from INIBAP *Musa* collection were received by the National Bureau of Plant Genetic Resources in New Delhi. The material, received as proliferating pre-rooted cultures, were regenerated into plantlets, hardened and established for field trials in Kennara to evaluate their agronomic performance and resistance to *Mycosphaerella* leaf spot diseases, weevils and nematodes.

The site, located at an elevation of 58 m, has an average temperature of 28^{o}C. The annual rainfall ranges between 2800 to 3050 mm and relative humidity varies between 77 and 94%. The performance of the hybrids was evaluated over two seasons (2001 to 2003). Plant height, pseudostem girth, number of functional leaves at bunch emergence, total crop duration, bunch weight, number of hands and fruits per bunch, fruit weight, fruit length and pulp-peel ratio were recorded. The infection index based on Gauhl's modification of Stover's severity scoring system and the youngest leaf spotted[1] were used to evaluate the resistance of the hybrids. Nematode resistance was determined by observing root and corm damage.

The hybrids displayed significant variation in vegetative characteristics with TMBx1378 being the tallest and SH–3640 the shortest plants. Bunch weight ranged from 16 kg in FHIA-18 to 36.5 kg in FHIA-25. FHIA-25 also had the highest number of hands and fruits. FHIA-21, CRPB-39 and TMBx5295-1, which have the pulp characteristics of a plantain, are suited for the preparation of chips. The hybrids TMBx5295-1, FHIA-18, FHIA-21 and CRPB-39 displayed very high resistance to leaf spot diseases. CRPB-39 showed susceptibility to pseudostem weevil (*Odoiporus longicollis*). Variation in the response to nematode infestation was also recorded.

1. INIBAP/IPGRI. 1998. Evaluation of *Musa* germplasm for resistance to Sigatoka diseases and Fusarium wilt. INIBAP Technical Guidelines 3. INIBAP, Montpellier, France.

Tissue culture as a strategy for maintaining biosecurity, enhancing diversity and delivering the benefits of biotechnology

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The Australian banana industry is entering a new era based on the demands of the consumer. Food quality, environmental impacts, increased pest and disease pressure and global competition are key issues. Research on tissue culture plays an important role in a biosecurity strategy and contributed to the ongoing research efforts directed at long-term sustainability issues regarding pests, diseases and markets. Australia's unique responses to banana germplasm maintenance and use will be discussed.

The Australian banana industry learned the importance of both domestic and international security from the devastating Fusarium wilt and banana bunchy top disease outbreaks of the early 1900s that were caused by an uncontrolled importation of infected planting material. The Australian industry has since put in place strict domestic and international quarantine standards to contain pests and diseases and prevent new introductions. Integral to this strategy is virus-free tissue culture planting material. Disease-indexed tissue culture plants is the only material that can be imported, moved or used in Australia and only accredited laboratories and nurseries located in disease-free locations can supply tissue culture planting material under permit.

The banana diversity that supports Australian research is maintained *in vitro* under reduced growth conditions. This collection of approximately 500 accessions, which began in 1985, is not a conservation collection but an active collection used to provide selected cultivars for research and industry development, and to provide disease resistant varieties to support disease exclusion programmes. Twenty plantlets of each accession are stored at 16^{o}C under low light, to reduce subculture periods. Although the majority of germplasm use is by Australian researchers, there are demands from Pacific nations to access this disease-indexed germplasm. Over the past seven years, approximately 120 000 plants have been supplied in response to almost 4000 requests for individual accessions. The Australian Quarantine Inspection Service registers entry point for banana varieties into Australia that are imported as tissue culture plants. Strict protocols to eliminate the risk of viruses escaping detection, combined with extensive quarantine post entry cultivation and inspection, ensure that no disease will be introduced via importation.

Some of the recognised problems with medium and long-term storage of *in vitro* germplasm are somaclonal variation, contamination and loss of vigour. To minimise these problems and maintain vigorous true-to-type plants we periodically reinitiate needed accessions. To support the tissue culture collection we maintain a field collection to determine true-to-typeness and to obtain good quality disease-free suckers. The field site is also used for agronomic evaluation and genotype characterization. All cultures are fully virus indexed. In Australia, banana tissue cultures are initiated following guidelines set by our Quality Banana Approved Nursery (QBAN) scheme to guarantee that tissue culture material is disease free. Source plants must be visually inspected as disease free and typical for the variety, suckers are collected and the leaves and upper pseudostem of each sucker are sent directly for virus indexing to the INIBAP *Musa* Indexing Centre at DPI's virology laboratory. The base of the sucker is trimmed and sent to the QBAN laboratory for initiation.

Our tissue culture research has provided a range of solutions in removing obstacles to the use of tissue culture such as: overcoming commercial multiplication problems caused by a limited multiplication phase, methods for roguing dwarf off types of the commercial Cavendish cultivars in the nursery and the use of molecular markers to detect off types in tissue culture and improve tolerance to *Fusarium oxysporum* f.sp. *cubense* subtropical race 4. Other research has identified problems such as increased susceptibility of tissue-cultured plants to soil borne disease.

Ongoing research is seeking ways to handle the sporadic reoccurrence of bacteria in medium-term reduced growth stores and to elucidate the molecular basis of somaclonal variation in banana tissue culture.

Somaclonal variant of ‘Valery’

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As part of a participatory research network conducted with banana planters, an off-type of a ‘Valery’ clone fas found in a commercial plantation. This plant was obtained by tissue culture propagation. The variety ‘Valery’ is susceptible to *Mycosphaerella fijiensis* but the micropropagated plant has shown a stable partial resistance to fungal infection over four generations in the plantation and is producing commercially acceptable fruits. This finding motivated a phenotypic, phytopathological and molecular evaluation of this promising somaclone.

The INIBAP descriptors were applied to the propagated commercial genotypes of Ecuador and to the off-type. The cluster analysis highlighted a significant phenotypic difference of the off-type plant, compared to the other commercial varieties. The most affected characteristic was the colour of the pseudostem.

In a greenhouse evaluation, the regenerated plants from the fifth *in vitro* generation are showing partial resistance compared to partially resistant and resistant reference genotypes. Further evaluation of the somaclone and the reference varieties is being conducted in the field under a high level of inoculum.

Isoenzymatic analysis of the clone and its related commercial varieties showed a different electrophoretic pattern of esterases and peroxidases. Highly pure total genomic DNA were analysed by PCR and RAPD techniques using commercial primers (OPJ-Operon kit) and the clone could be differentiated from the related varieties. These results were statistically confirmed by the determination of the coefficient of similitude between the electrophoretic pattern distribution represented in a dendogram.

In order to understand the possible mutation influencing the pseudostem colour of the clone, restriction analysis of chloroplastic DNA was performed with vitroplantlets and field plants. The phenotypic, phytopathological and molecular data of are presented and discussed.

Resistance to Fusarium wilt in irradiated 'Manzano' (AAB) and 'Gros Michel' (AAA)

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The objective of this project was to select for somaclones resistant to race 1 and 2 of Fusarium wilt (caused by *Fusarium oxysporum* var. *cubense*) among irradiated plants of the susceptible 'Manzano' (AAB) and 'Gros Michel' (AAA) cultivars. Vitroplants regenerated from irradiated adventitious buds of both cultivars were inoculated with isolates of the fungus. After many cycles of selection in nurseries and in heavily infested fields, the selected clones were planted in field 32. These were evaluated over two production cycles and 9 'Gros Michel' somaclones with an infection index inferior to 30% were selected. All the material derived from 'Manzano' was classified as susceptible after the first production cycle. The resistant somaclones that had the desirable agronomic characteristics were micropropagated *in vitro* and evaluated for their yield. Three somaclones with promising characteristics have been selecting.

Potential of phytocystatins in the transgenic control of banana weevil (*Cosmopolites sordidus*)

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Phytocystatins are proteins of plant origin that reversibly inhibit cysteine proteases. They occur widely in plants and have been reported to contribute to natural host plant resistance against disease and pest organisms. Cysteine proteases are enzymes that break down dietary proteins, mainly in the guts of coleopteran insects such as the banana weevil (*Cosmopolitus sordidus*). This makes them potential tools for the control of coleopteran insects in transgenic plants. Studies were undertaken to investigate the potential of using phytocystatins in the control of banana weevil.

Using *in vitro* assays, gut extracts from larvae were exposed to specific fluorescent protease substrates in order to detect protease activity. This was followed by inhibition of substrate hydrolysis using synthetic inhibitors and recombinant phytocystatins. Genes of phytocystatins from rice and papaya were successfully cloned into commercially available expression vectors, and their cystatins were expressed in *E. coli* and purified from bacterial cultures. Banana weevil larvae extracts contained significant amounts of cysteine and serine proteases. The activity of the cysteine proteases was highly inhibited by recombinant produced rice and papaya cystatins, both in fluorimetric assays and gelatin-containing polyacrylamide gel electropohoresis. Phytocystatins might, therefore, have the potential to control banana weevil and other coleopteran pests in transgenic plants. However, protease engineering and pyramiding of inhibitory genes might be required to improve pest control.

Genetic transformation using chimeric antifungal genes

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Bananas and plantains are difficult crops to breed because most of the important cultivars are highly sterile and seedless. The use of genetic transformation presents an alternative for the development of resistance to diseases such as black leaf streak disease.

Transgenic banana plants have been obtained by *Agrobacterium*-mediated transformation of embryogenic cell suspensions[1]. In this work, we describe the *Agrobacterium*-mediated transformation of 'Grand naine' (AAA) and 'Navolean' (AAB) using chimeric genes of antifungal proteins.

Immature flowers[2] and scalps were respectively used as starting material to obtain embryogenic cell suspensions of 'Grand naine' and 'Navolean'. The EHA-105 strain of *Agrobacterium tumefaciens* carrying the plasmids pHCA58, pHCG59 and pHGA91 (C - chitinase gene, G - β-glucanase gene, A - AP24 gene), was used. Genetic transformation was carried out as described in Pérez[1], with the exception that co-cultivation lasted four hours. The culture media used at each stage were: semisolid multiplication medium M2[2] for 'Grande naine' and ZZI[3] for 'Navolean' during co-cultivation; the same media supplemented with 200 mg/L of timentine and 6 mg/L of Basta during selection; M3[2] and RD1[3] during embryo formation; and the medium described by Gómez *et al.*[4] during germination.

After three months of selection, globular stage embryos appeared on the surface of the embryogenic cells cultured on M3 and RD1. The mature embryos were transferred to the germination medium. Coleoptiles emerged after 4 to 8 weeks. Several putative transgenic lines have been regenerated, multiplied and acclimatized for planting in the field (Table 1).

Evidence of stable integration of the transferred genes in the genome of the regenerated plants was obtained by PCR analysis (Figure 1) and Southern analysis using primers and probes specific to the *bar* gene. Preliminary Southern hybridizations have revealed a low copy number for the incorporated *bar* gene. Further molecular analysis is in progress.

After obtaining the permission of the National Biosafety Committee, 117 transgenic lines were planted in the field for evaluation against black leaf streak disease. A completely random design was used, with three replicates per line and three plants per replicate. Untransformed lines were used as controls and as border plants. The first results of the evaluation are presented.

1. Pérez J.B. 2000. Development and application of *Agrobacterium*-mediated genetic transformation to increase fungus-resistance in banana (*Musa spp.*). Doctoraatsproefschift Nr. 442 aan de Faculteit Landbouwkundige en Togepaste Biologische Wetenschappen van de K.U.Leuven, Belgium.

2. Côte F., R. Domergue, S. Monmarson, J. Schwendiman, C. Teisson and J.V. Escalant. 1996. Embryogenic cell suspensions from male flower of *Musa* AAA cv. "Grand Naine". Physiol Plant 97:285-290.

3. Dhed'a D., F. Dumortier, B. Panis, D. Vuylsteke and E. De Langhe. 1991. Plant regeneration in cell suspension cultures of the cooking banana cv. "Bluggoe" (*Musa* spp. ABB group). Fruits 46:125-135.

4. Gómez R., T. Gilliard, L.A. Barranco and M. Reyes. 2000. Embriogénesis somática en medios líquidos. Maduración y aumento de la germinación en el cultivar híbrido FHIA-18 (AAAB). INFO*MUSA* 9(1):12-16.

Table 1. Status of different phases of genetic transformation.

Cultivar	Construct	Number of transformed samples	Mean number of colonies per sample	Number of shoots regenerated	Number of plants in acclimatization	Number of plants in the field
Grande naine	pHCA58	6	8.4	45	21	14
Grande naine	pHCG59	12	6.3	68	41	35
Grande naine	pHGA91	6	3.4	24	21	20
Navolean	pHCA58	9	5.4	80	33	21
Navolean	pHGA91	12	9.3	104	33	28

Figure 1. *PCR analysis of putatively transformed 'Navolean' plants. Lanes 1-8: transformed lines; Lane 9: negative control (untransformed plant). Lane 10: positive control (pHCA58). Lane 11: 1-Kb molecular weight ladder (Gibco).*

Genetic transformation of cv. 'Grande naine'

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Yield loss due to pests and diseases, in particular black leaf streak disease, which has increased considerably in recent years, is now considered the most significant factor affecting banana production worldwide. Agrichemicals have been used to control many pests and diseases, but as the economical and environmental costs continue to rise, there is a growing need for resistant cultivars as part of an integrated system for pest management. In this context, the genetic manipulation of banana with appropriate antifungal genes represents an interesting approach to generate resistance to fungal disease.

We have established embryogenic cell suspensions from immature male flowers of cv. 'Grande naine' and, by using biolistics, have transferred several genes coding for antifungal proteins. The genes employed include chitinase, glucanase and one antifungal gene from *Capsicum anuum* under the control of a constitutive promoter. The regenerated plants have now been in the field for two years. They have been subjected to different analyses to determine their tolerance/resistance to black leaf streak disease, both in the greenhouse and the field. The results of these evaluations will be discussed.

In addition, several genes coding for human antigens have been introduced to cell suspensions by using biolistics and transformed plants have been regenerated. The genes employed include a string of epitopes from *Plasmodium falsiparum*, some antigens from HIV and one antigen from the rabies virus. The potential of the fruits of these plants to be used as edible vaccines will be discussed.

4th International symposium on the molecular and cellular biology

Keynote lecture: Can model plants help improve bananas through biotechnology?

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Genetic engineering for enhanced agronomic traits holds considerable promise and experimental power, but has registered varying degrees of success. Bananas are particularly well suited for this approach, given the serious limitations inherent to conventionally breeding this crop, such as low female fertility, sterility, poor seed set and the triploid nature of the genome of commercial varieties. While possible, classical breeding is also time consuming. Taking into consideration these constraints, alternative strategies are needed. The banana transformation technology is sufficiently advanced to consider feasible improving banana by genetic engineering. Two key questions regarding "molecular breeding" of banana will be addressed. 1) Which genes and approaches have a realistic chance to be effective? 2) And can we extrapolate useful information from model plants to bananas?

*Arabidopsi*s has been an invaluable model system to study many aspects of plant biology, including plant pathology and plant stress physiology, with many insights directly applicable to crop plants. In addition *Arabidopsis* has a number of experimental advantages: the genome is sequenced, microarray chips are available and there are a multitude of well-characterized mutants. A discussion of the pros and cons of using the *Arabidopsis* model will be presented as well as the possibilities of using as a model for crop improvement a considerably closer relative of banana, rice.

Finally, our work on the modulation of plant programmed cell death will be presented. A number of plant diseases caused by necrotrophic fungi as well as abiotic stresses (heat, cold, salt, and drought) require the induction of a plant apoptotic-like programmed cell death response. We have shown that expression of cell death modulating genes from diverse sources are effective in conferring heritable tolerance/resistance in tobacco, *Arabidoposis*, tomato and wheat. These data will be discussed in the context of our efforts in inserting "anti-death" genes in banana with particular emphasis on black leaf streak disease and Fusarium wilt.

Comparative analysis of *Musa* and rice genome structure and organization

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Within the *Musa* Genomics Consortium three BAC libraries were constructed from the genomes of *Musa acuminata* (cvs 'Calcutta 4' and 'Grande naine') and *Musa balbisiana* (PKW). The three BAC libraries have been anchored to the existing *Musa* consensus genetic map using a set of single-copy RFLP markers. The three BAC libraries are a publicly available resource and represent a critical tool to achieve the goals of the recently created Global *Musa* Genomics Consortium.

To study the structure and evolution of *Musa* genomes in relation to those of rice and sorghum, 50 *Sorghum bicolor* cDNA clones, previously mapped in sorghum and rice with large-hybridization spectrum (EGRAM genes), were used as probes to screen the *M*. *acuminata* 'Calcutta-4' BAC library. Banana BACs that hybridized to 9 EGRAM probes of known function were further analysed by BAC end sequencing and RFLP fingerprinting, and individual BAC clones were selected and subcloned for complete sequencing. In addition, BAC clones believed to arise from the homoeologous region on the A and B *Musa* genomes were identified using *M. acuminata* RFLP genetically mapped probes encoding for genes of agronomic interest and involved in plant defense (1-3 glucanase) and control of plant height (GA 20-oxidase).

Altogether, 13 *Musa* BACs have been sequenced, totalling over one Mb. A preliminary analysis showed that the gene density in the sampled *Musa* genomic regions was approximately one per 5 kb and that coding regions are 65-75% similar to those from both rice and *Arabidopsis*. The relationship between rice and *Arabidopsis* genomes and the gene content and organization of the sequenced banana regions the will be reported.

The first insights provided by the international *Musa* sequencing project cover:

1. the relationship between duplicated regions within the *M*. *acuminata* genome,
2. the comparison of orthologous genomic regions in rice and banana,
3. the conservation of homoeologous regions in *M*. *acuminata* and *M*. *balbisiana.*

These data will shed some light on the relationships, at the microsynteny level, between chromosomal regions of agronomic interest for banana breeding in distantly related monocot species.

Identification of *Musa* chromosomes by fluorescence *in situ* hybridization with BAC clones

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The analysis of the *Musa* karyotype is hampered by poor morphological differentiation and the small size of the chromosomes. Fluorescence *in situ* hybridization (FISH) with probes for ribosomal DNA has been shown to be useful to identify some chromosomes. The 45S rDNA provided the first and until now the only chromosome-specific molecular marker in *Musa* while the 5S rDNA can be used to detect a subgroup of chromosomes. As the isolation of repetitive DNA sequences did not result in chromosome-specific landmarks, isolating chromosome-specific clones from BAC (bacterial artificial chromosome) libraries seems to be the most promising route. The aim of this work was to develop a set of chromosome-specific cytogenetic markers for bananas using FISH with labelled BAC clones.

Seventy anonymous BAC clones were selected for their low content of repetitive DNA after screening two genomic libraries of *Musa acuminata* and ten clones were selected from the BAC library of *Musa balbisiana*. The amount of repetitive DNA sequences within the BAC clones varied substantially, resulting in FISH signals with different distribution and specificity along the chromosomes. Based on the distribution, specificity and intensity of the hybridization signals, the probes were classified in four groups. The first group of BAC probes showed multiple sites of hybridization clustered as tandem repeats. In the second group, the signals were distributed almost uniformly along the chromosomes, with a tendency to cluster in broad centromeric regions. The third group of BAC clones probably contained large amounts of repetitive DNA and showed genomic-like hybridization pattern with signals covering entire chromosomes. The fourth group of BAC probes was characterized by dispersed hybridization over the banana genome with gaps near centromeres and nucleolar organizer regions.

FISH revealed distinct chromosome signals only with the first two groups of BAC clones. In some BAC clones, the suppression of repeated DNA could be achieved by pre-annealing excess amounts of unlabelled C_0t-1 fraction with denatured chromosomes before the addition of the labelled probe. Low and single copy BAC clones were selected and, along with ribosomal DNA probes, they facilitated the identification of all eleven chromosomes of *M. acuminata*. Using FISH with BAC clones differing in the amount of repetitive DNA revealed gene-rich and gene-poor chromosome regions. The possibility of localizing BAC clones selected from *M. acuminata* on chromosomes of *M. balbisiana* (and vice versa) opens the way for comparative physical mapping. It is expected that the identification of individual chromosomes using physically mapped DNA sequences will permit the analysis of their behaviour and segregation during evolution and in breeding programmes. Physically mapped single and low-copy DNA sequences will also provide the anchor sites needed to integrate the physical and genetic maps.

The BAC libraries of M. acuminata *were obtained from CIRAD and CICY. This work was supported by the Academy of Sciences of the Czech Republic (grant no. A6038201) and the International Atomic Energy Agency (Research Contract No. 12230/RBF).*

Flow cytometry and chromosome analysis of *Musa* accessions from Papua New Guinea

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Knowledge of the ploidy level of banana cultivars is important for banana breeding. Although most cultivated bananas are triploid (2n=3x=33), diploid and tetraploid bananas also exist. In this study, we used flow cytometry and chromosome counting to assess the ploidy levels of 18 *Musa* accessions from Papua New Guinea. Flow cytometric analysis was done with a Partec CA flow cytometer (Partec, Munster, Germany). Nuclei were isolated by chopping 20-30 mg of midrib tissue with a sharp razor blade in a plastic petri dish containing Otto I buffer (0.1 M citric acid and 0.5% Tween 20). The suspension was filtered through a 40 um nylon mesh. The nuclei were stained in Otto II buffer (0.4 M sodium hydrogen phosphate) containing DAPI. The DNA content was determined by observing the peak positions of the histograms. Prior to the analysis, the gain of the instrument was set so that a diploid peak appeared at channel 50.

Chromosome counts were prepared from young root tips. Actively growing root tips were pretreated in 0.036% 8-hydroxyquinoline for 2 hours a room temperature, fixed in ethanol-acetic acid (3:1), digested with an enzyme mixture and spread on a glass slide. The chromosomes were stained with Giemsa and photographed. Twelve of the 18 accessions were diploid and the rest were triploid. New chromosome numbers were determined for five of the accessions: ‘Ambiri’, ‘Bagul’, ‘Gunih’, ‘Marau’ and ‘Pitu’. ‘Ambiri’ and ‘Marau’, previously reported as triploids, were found to be diploids in this study, whereas ‘Bagul’, ‘Gunih’ and ‘Pitu’, previously reported as triploids, were found to be diploids. The chromosome number of the accession ‘Gia hui’ (2n=2x=22) was reported for the first time. No correlation was found between the average number of chloroplasts in guard cells and ploidy level. The average chromosome size ranged from 1.1 to 2.1 µM, suggesting that species evolution in *Musa* was also accompanied by concomitant changes in chromosome size.

Chloroplast DNA polymorphism in Asian cultivars

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It is widely believed that edible seedless banana cultivars are derived from or are hybrids of two wild diploid species, namely *Musa acuminata* Colla (AA) and *Musa balbisiana* Colla (BB). Domestication, believed to have occurred in southeast Asia, resulted in better quality cultivars for human consumption. However, the geographical and genomic origins of the hybrids are still uncertain.

We explored four chloroplast regions, *rpl*16, *psa*A, *ndh*A, and *pet*A, and detected single strand conformation polymorphisms (SSCPs) in wild species and hybrid accessions. We selected 18 haplotypes: 5 BB, 2 BBB, 4 ABB, 1 ABBB, 4 AA and 2 AAA. Three Australimusa *Musa Fehi*, *Musa jackeyi* and *Musa textiles*, were used as outgroup taxa. Thirty polymorphisms in the 4857 bp combined sequence data were found between the A and B types, six within the A haplotypes and five within the B haplotypes. The polymorphisms separating the two species included single nucleotide substitutions, single nucleotide indels and ten large insertion/deletion events. The polymorphisms within each species included single nucleotide substitutions, length variation in four mononucleotide repeats and one large insertion/deletion.

Based on these data, haplotype networks were constructed. The result indicated that the hybrid cultivar ‘Namwa’ from Thailand did not originate from the wild *M. balbisiana* found in northern Thailand. The A chloroplast haplotypes were more closely related to *M. acuminata* ssp. *malaccensis* than to ssp. *siamea*. Some triploid hybrids contained A chloroplasts (AAB/ABB) and some contained B chloroplasts (BBA/BBB). The tetraploid hybrid ‘Pli hai’ contained B chloroplasts (BBBA). The chloroplasts of all ‘Namwa’ (BBA) had the same wild origin but the parents are still unknown. The ‘Namwa’ chloroplast does not match any wild *M. balbisiana* from the Philippines, Thailand, the Inibap Transit Centre in Belgium or the Australimusa species studied.

Characterization of translocations in 'Calcutta 4' and 'Madang'

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Chromosome pairing studies at meiosis have revealed that translocations are frequent in banana genomes. In *Musa acuminata*, seven groups of translocation have been identified (Standard, North Malaysia, Malaysia Mountains, North A, North B, Indonesia, and East Africa). Each group comprises accessions having the same chromosome structure. Translocations increase the difficulty of constructing a genetic map, studying the inheritance of agronomical characters and breeding in general. The objectives of our study were to develop a tool to characterize translocations on chromosomes based on fluorescent *in situ* hybridization of BAC clones (BAC-FISH) and to use this tool to characterize the translocations in the accessions 'Calcutta 4' (2n=2x=22, translocation group North A) and 'Madang' (2n=2x=22, translocation group Standard).

To achieve this, a genetic map and a 'Calcutta 4' BAC library were built, the BAC-FISH technology was adapted to bananas and a banana cytogenetic map was initiated. The genetic map was based on a F2 population from a 'Calcutta 4' x 'Madang' cross. It encompasses 120 markers (20 RFLPs, 81 AFLPs and 19 SSR markers) distributed over 14 linkage groups and covering 597 cM. The 'Calcutta 4' BAC library comprises 55 152 clones with an average insert size of 100 Kb. About 1.5% of these clones present chloroplast and mitochondrial DNA inserts. This library covers 9 to 10 times the banana genome.

Linkage group II (LGII) was chosen to search for translocations since some of its characteristic (such as a high number of distorted markers) suggested that this group contained chromosomes with translocations. Four BAC clones corresponding to 3 RFLPs and 1 SSR loci distributed on LG II were localized on the 'Calcutta 4' and 'Madang' chromosomes. The results showed that the markers involved in the LG II were localized on three chromosomes, whose structure was different in 'Calcutta 4' and 'Madang', with two linked translocations observed in 'Calcutta 4'. In 'Madang', the markers mMaCIR161-rMaCIR 560, rMaCIR 1125 and rMaCIR 36 were localized on three chromosomes (A, B and C). In 'Calcutta 4' these markers were localized on only two chromosomes (A and B). The marker rMaCIR 1125 localized on the 'Madang' C chromosome, was localized in the proximal position of the 'Calcutta 4' B chromosome. We also initiated a global cytogenetic map of 'Calcutta 4' that comprises 16 loci (14 BAC clones selected by RFLP and SSR markers and the ribosome probes 45S and 5S). The 14 linkage groups of the 'Calcutta 4' x 'Madang' genetic map are anchored to this cytogenetic map.

Gene content and density in two *Musa acuminata* BAC clones

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Two BAC clones from a *Musa acuminata* (Calcutta 4) C4-Bam BAC library were randomly selected for sequencing by restriction analysis and the BAC DNA was fragmented by nebulization. Fragments of the desired length were purified through high performance electrophoresis chromatography, subcloned in pUC18 and sequenced. The analysis of the first BAC (MuH9, 82.723 bp) revealed 12 putative protein-coding regions, representing a gene density of one gene per 6.9 Kb. This is slightly less than the reported gene density for *Arabidopsis*[1] but similar to the one for rice[2]. The functions of two of the putative proteins could be deduced: malate synthase and the crinkly-4 transcription factor. The others were similar to predicted proteins identified in genome sequence databases. Only 7 coding regions were discovered in the second BAC (MuG9, 73.268 bp), for an overall gene density of one gene per 10.5 Kb. One coding sequence displayed significant homology to ribonucleotide reductases (large subunit) whereas the others were homologous to hypothetical proteins. A transition area between coding regions and repeated sequences was found at around 54 Kb. It separated the gene-dense 5' end from the 3' end and mainly contained retrotransposon-like sequences and repetitive sequences known in *M. acuminata*. This gene organization resembles the one of Gramineae genomes, in which genes are clustered in gene-rich regions separated by gene-poor DNA containing abundant transposable elements, and differs from the one of *Arabidopsis* and rice genomes (chromosomes 1 and 4), in which genes are fairly evenly distributed.

1. The *Arabidopsis* Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796-815.

2. Feng *et al*. 2002. Sequence and analysis of rice chromosome 4. Nature 420:316-319.

Isolation, cloning and characterization of fruit expressed β-galactosidase and polygalacturonase cDNAs

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Bananas are economically important tropical fruits that rapidly soften during ripening. The fruits usually have a post-harvest life of about one to two weeks. Several cell wall hydrolases are implicated in the softening of the fruit[1, 2, 3, 4]. The major cell wall hydrolases involved in fruit softening are β-galactosidase and polygalacturonase. The role of β-galactosidase is important, as its activity may be necessary to allow several other cell wall hydrolases, including polygalacturonase, to access their sites of action. In the course of our studies on the molecular events during fruit ripening of cv. 'Robusta', we have isolated putative fruit expressed β-galactosidase and polygalacturonase cDNAs.

Degenerate primers specific for conserved regions of β-galactosidase and polygalacturonase were synthesized based on their respective sequences, available in the NCBI and EMBL databases. Total and mRNA were obtained from 25% and 50% ripe fruits and an RT-PCR was performed using the designed degenerate gene specific primers. Amplified cDNAs were eluted and cloned into a pTZ57R vector. These cloned cDNAs were sequenced and characterized using NCBI's BLAST (X) programme.

The cDNAs isolated using the β-galactosidase gene specific primers had 846 bp and encoded for 283 amino acids open reading frame, the deduced sequence of which was similar to other fruit β-galactosidases. The nearest match was to the β-galactosidase of *Asparagus officianalis* (Accession No. CAA54525), which was 77% similar, or 84% similar when conserved substitutions were allowed. The cDNA isolated using the polygalacturonase gene specific primer had 715 bp and encoded for 178 amino acids open reading frame, which is the 3' end of the gene with a stop codon at the 535th bp (179th aminoacid) and a 180 bp 3' untranslated region along the poly A tail. The deduced sequence was similar to that of other fruit polygalacturonases. The best match was to the polygalacturonase of *Oryza sativa* (Accession No. BAB55744), which was 77% similar, or 88% similar when conserved substitutions were allowed.

The β-galactosidase and polygalacturonase cDNAs are being used as probes in expression studies during fruit ripening. Their full-length sequences are also being isolated to use in the potential development of transgenic bananas with increased shelf life.

1. Brummell D.A. and M.H. Harpester. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Molecular Biology. 47:311-340.

2. Giovannoni J. 2001. Molecular biology of fruit ripening. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52:725-749.

3. Marin-Rodríguez M.C., J. Orchard & G.B. Seymour. 2002. Pectate lyases, cell wall degradation and fruit softening. J. Exp. Bot. 53:2115-2119.

4. Smith D.L., J.A. Abbott & K.C. Gross. 2002. Down-regulation of tomato b-galactosidase 4 results in decreased fruit softening. Plant Physiol. 129:1755-1762.

Diversity of LTR retrotransposons and their use as markers

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Phylogenetic analyses have demonstrated that most LTR-containing retrotransposons belong to one of two subgroups, *pseudoviridae* and *metaviridae* (traditionally Ty1*/Copia* and Ty3*/Gypsy*), and are recognized by the presence of reverse transcriptase (RT) domain sequences in their genome. In order to understand the genomic organization of LTR retrotransposons in the banana genome, we used partial RT sequences of LTR retrotransposons cloned from various banana varieties as probes for fluorescent *in situ* hybridization (FISH). Microarray analysis was used to study the abundance of LTR retrotransposons in the different banana genomes. Inverted-primer polymerase chain reaction (IP-PCR) was used to study the insertion patterns of LTR retrotransposons and then further developed for use as a marker system to help banana breeders.

FISH showed that both element classes are present in all chromosomes of the varieties, with different signal intensity, and are located in the centromeric region. There are colocalized sites on some banana chromosomes for these two LTR retrotransposon subgroups. Microarray analysis of LTR retrotransposon clones showed that the copy number of LTR retrotransposons varies with the subgroup and genome constitution. Sequence analysis of the IP-PCR products showed that there are two main insertion events of LTR retrotransposons in the banana genome. Insertion can happen in gene-rich regions. LTR retrotransposons inserted in the coding region of certain genes might disrupt the functions of that particular gene. Insertion can also happen in gene-poor regions. LTR retrotransposons inserted in an intergenic region may or may not affect the function of the genes that are near the insertion sites. Hybridization of the products from this insertion showed that the pattern of these events is independent of the genome constitution.

Characterization of promoter tagged lines

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We have developed a fast, sensitive and high-throughput method for the identification and isolation in bananas of novel promoters and genes. Following the *Agrobacterium*-mediated transformation of embryogenic cell suspension cultures with a promoterless luciferase (*luc*) gene, candidate promoters are characterized *in planta* during the early stages of *in vitro* development. Screening for luciferase (LUC) enzymatic activity in transgenic cell colonies has been performed without induction treatments. We have previously reported that transformation of a luciferase reporter trap linked to the left T-DNA border resulted in an activation frequency of 0.03-0.07%.

All tagged candidates have now been propagated for plant regeneration and molecular analysis. Southern hybridization and sequence analysis of several independent lines demonstrated the integration of 2-6 T-DNA copies in the genome with the frequent presence of the vector backbone or complex rearrangements. Therefore, TAIL-PCR products from selected tagged lines were individually cloned and sequenced.

One tagged line displayed constitutive expression higher than that of the CaMV 35S promoter, which persisted in the regenerated plants, both *in vitro* and in the greenhouse. Detailed analysis of the LUC expression in all plant tissues will be presented. One of the T-DNA insertions in this line occurred in a metallothionein-like protein gene, demonstrating the feasibility of the technique with bananas.

An improved tagging construct, which contains the codon-optimized *luc*$^+$ gene fused close to the right T-DNA border, has been prepared. Transformation with this construct resulted in a 40-fold increase in activation frequency (up to 2.5% without induction) compared to the original tagging construct. Data on LUC expression in hundreds of independent tagged lines will be presented. This T-DNA trapping technology is currently being applied to isolate inducible genes and promoters.

The role of intermediates and shunt metabolites of the melanin pathway in the virulence of *Mycosphaerella fijiensis*

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Melanin shunt metabolites, including juglone, 2-hydroxyjuglone, flaviolin, 4-hydroxyscytalone and 2,4,8-trihydroxytetralone, have been predicted to serve as toxins in black leaf streak disease (BLSD) caused by *Mycosphaerella fijiensis*. Previous researchers demonstrated that purified toxins and fungal culture extracts applied to susceptible leaves caused necrotic symptoms similar to those caused by the fungus. Furthermore, juglone or crude fungal extracts have been used to select banana genotypes resistant to fungal phytotoxins, with the hypothesis that such metabolite-resistant clones would exhibit increased resistance to *M. fijiensis*. To date, however, there has been no clear demonstration that such a selection process results in increased resistance to the fungus. Furthermore, genetic evidence that a particular metabolite or metabolic pathway contributes to pathogen virulence has been lacking. This is primarily because tools for the molecular genetic manipulation of *M. fijiensis* have not been available until recently. Since the role of toxins is unclear, a molecular genetics approach was adopted to determine whether the metabolites involved in melanin biosynthesis and the shunt products of the pathway play a role in BLSD.

Fragments of 22 genes were cloned by PCR amplification of products from *M. fijiensis* DNA with degenerate primers designed to conserve regions of known proteins. These genes include several purported to be involved in melanin biosynthesis, the production of other secondary metabolites (sugar, toxin and peptide transporters), and in fungal development and phytoalexin detoxification. One of these genes, *PKS1*, is a polyketide synthase gene expressed *in planta* that is highly similar to other genes involved in the first step of fungal melanin biosynthesis. We used *Agrobacterium*-mediated transformation to knock out the function of *PKS1* by homologous recombination and confirmed by molecular analyses that the gene was disrupted. Knockout transformants purified to genetic homogeneity are stably deficient in melanin production, i.e., cream-colored in comparison with the isogenic black wild type strain. We will discuss our results characterizing the growth of these melanin-deficient knockout mutants *in vitro* and *in planta*, including chemical analyses and pathogenicity assays of wild type and mutant strains.

Gene expression profiling in leaves infected with *Mycosphaerella fijiensis*

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Complex physiological changes are associated with pathogenesis, many of which are preceded by alterations in gene expression. We used Serial Analysis of Gene Expression (SAGE) to produce a quantitative analysis of gene expression in leaves infected with *Mycosphaerella fijiensis*, the causal agent of black leaf streak disease. For this purpose, we applied an improved SAGE protocol that generates 26-bp tags instead of the standard 13-bp tags.

A total of 20 392 tags were sequenced from two SAGE libraries generated from field-infected and non-infected leaves of a resistant *Musa acuminata*. The total number of unique tags was 6735 and 5292 for the infected and non-infected library, respectively. These tags were defined as different genes.

A total of 163 putative differentially expressed genes were identified by *in silicio* comparison between different genes obtained from infected and non-infected leaves, demonstrating the power of SAGE for identifying differentially expressed genes in banana. Of these, 96 genes were repressed and 67 genes were induced in leaves infected with *Mycosphaerella.* Only 79 (48%) of the putative differentially expressed tags matched EST sequences in databases. An overview of the functional annotation for the differentially expressed tags as well as the most abundant transcripts will be shown.

Since a large number of banana tags identified by SAGE did not match EST sequences in databases, unknown tags were used as forward primers for 3' RACE. This allowed the recovery of an extended sequence for the most repressed (91-fold) tag. Sequence analysis revealed significant homology to a glycine-rich RNA binding protein.

Microarray analysis of gene expression using plants infected with *Fusarium oxysporum* f.sp.*cubense*

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Fusarium wilt, primarily caused by *Fusarium oxysporum* f.sp. *cubense*, is a destructive disease causing massive production loss of commercial cultivars worldwide. To date, no effective strategy has been found to combat this disease. With large-scale sequencing and gene analysis becoming more available, global analysis of gene expression in response to Fusarium infection can be used. In this study, cDNA libraries for 'Mutiara' (AAB), which is tolerant to Fusarium, and for 'Rastali (AAB), which is susceptible to Fusarium, were constructed.

Five micrograms of mRNA from each variety was used in the construction of their respective cDNA library and 4000 clones from each library were randomly cored and amplified using PCR. The clones were then arrayed on glass slides. The gene expression of the expressed sequence tags (ESTs) is being analysed. The results of this research may elucidate the pathways involved in the resistance and susceptibility to Fusarium infection and provide insights on the genetic mechanisms of disease tolerance in 'Mutiara'.

Potential resistance gene against Fusarium wilt race 4

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Fusarium wilt is a devastating disease of banana that has been recorded in all banana growing areas. Generating resistance to *Fusarium oxysporum* f.sp *cubense* in susceptible banana cultivars is the ultimate aim of many laboratories studying resistance (R) genes of *Musa*. The nucleotide-binding site, leucine rich repeat (NBS-LRR) type of R-gene is the primary focus of transgenic strategies due to their known interaction with pathogen virulence factors and elicitor molecules. In the Plant Biotechnology Research Program at QUT, we have been isolating and characterizing R-genes from a population of the diploid AA cultivar *Musa acuminata malaccensis* that have shown segregation for resistance to *Fusarium oxysporum* f sp *cubense* race 4.

Degenerate primers complementary to the NBS region were used in PCR reactions to target the central portion of the intron-less banana NBS-LRR genes from genomic DNA isolated from resistant plants. Five NBS regions were isolated and sequenced. Southern-blot hybridization analysis showed each of the five resistance gene candidates (RGCs) to be present as single copies in the genome. Transcriptional activity was assessed by reverse-transcriptase-PCR experiments, as northern analysis often fails to detect R-gene transcripts due to the low level of constitutive transcription of this class of genes in different plant species and tissues. One NBS-LRR gene (RGC-4) did not appear to be transcribed in either susceptible or resistant *M. a. malaccensis* plants while three genes, RGC-1, -3 and -5, were transcribed in both types of plants segregating in the population. Surprisingly, transcripts of RGC-2 were detected in resistant plants but could not be amplified from the mRNA of susceptible plants. Similarity searches of the GenBank database using BLAST algorithms showed the NBS region of this banana gene to have significant similarity and be most similar to the disease resistance I2 gene from tomato *Lycopersicon* that confers resistance to race 2 of the soil-borne fungus *Fusarium oxysporum* f sp *lycopersici*. The 5' and 3' regions of the banana RGC-2 were isolated by 5'-RACE and 3'-RACE, the regions sequenced and then the entire gene isolated in PCR experiments using primers specific for the 5' and 3' untranslated regions. Transgenic plants with the RGC-2 gene are currently being generated with a view to ascertain whether this gene confers resistance to *Fusarium oxysporum* f sp *cubense.*

Risk assessment of spreading BSV through *in vitro* culture

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In vitro multiplication is often reported as triggering the production of episomal infectious BSV particles, in hybrids harbouring all or part of the B genome, through the activation of integrated BSV endogenous pararetrovirus (EPRV) sequences. Nevertheless, mass production of vitroplantlets remains the most widely used method for distributing *Musa* germplasm and improved hybrids. A better understanding of the effects of *in vitro* culture on BSV EPRV activation is necessary to evaluate the risks of spreading BSV through the distribution of mass produced banana plantlets. In order to evaluate this risk, CIRAD and INIBAP have initiated a collaborative project aimed at answering the following questions:

- Does BSV EPRV activation occur through *in vitro* culture in all inter-specific hybrids and plantain cultivars?
- Is there a correlation between the number of *in vitro* subcultures and the percentage of plantlets exhibiting BSV episomal particles?

In order to answer these questions, three accessions were used: two plantain cultivars, ‘Kelong mekintu’ (AAB) and ‘Black penkelon’ (AAB), and the hybrid CRBP-39 (AAAB). Virus-free suckers were selected and mass propagated using standard *in vitro* budding methods. During the successive multiplication (proliferation) subcultures, at least 40 shoots were randomly picked and screened for the presence of episomal BSV particles, using standard immuno-capture PCR-based detection methods.

BSV episomal particles were detected in the plantain cultivars and the CRBP-39 hybrid, with BSV-Ol being the predominantly detected BSV strain. The plantain cultivars and CRBP-39 displayed similar patterns of activation. The percentage of plantlets positive for BSV-Ol rapidly increased after the first subculture cycles. Depending on the cultivar, the highest percentage of BSV-Ol positive plantlets ranged between 10% (‘Black penkelon’ and CRBP-39) and 20% (‘Kelong mekintu’). These percentages were obtained when the total number of produced shoots was between 800 and 2000. The percentage of BSV-Ol positive plantlets decreased as the number of subcultures increased. This was especially striking with CRBP-39, for which values below the detection threshold were obtained when the total number of produced shoots reached and exceeded 4000. The decrease was less pronounced for the two cultivars. The impact of these results on the *in vitro* mass propagation of *Musa* germplasm and plantlet distribution will be discussed.

Replication of banana bunchy top babuvirus and the rational design of resistance transgenes

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Strategies for generating transgenic resistance to plant RNA viruses have been highly successful, particularly the strategy of RNAi or posttranscriptional gene silencing. Unfortunately, these same strategies appear to be significantly less effective for generating resistance to plant ssDNA viruses (*Geminiviridae* and *Nanoviridae*), of which the banana bunchy top babuvirus (BBTV) is one. For these viruses, the most promising strategies are those that interfere with replication, particularly transdominant negative strategies involving constitutive over-expression of mutated Rep proteins (replication initiator protein) and a strategy developed by us involving a Rep activated cell death transgene. Importantly, these strategies are dependent upon the interaction of the Rep protein and the viral intergenic region. This interaction is highly specific and may well define the breadth of virus resistance that is generated using these approaches.

The rolling circle replication mechanism of plant ssDNA viruses has been best studied in the Begomovirus genus of the *Geminiviridae*. In these viruses, the Rep protein appears to recognise and bind to specific sequences within the intergenic region known as iterons. The Rep protein then nicks within the highly conserved loop sequence of the stem/loop origin of replication, thus initiating rolling circle replication. Motifs within the Rep protein have specific functions including binding, nicking and ligation. Thus, it is essential to have defined the sequences involved in plant ssDNA virus replication both at the nucleotide level in the intergenic region and the amino acid level in the Rep protein. Such information for members of the *Nanoviridae* has previously only been inferred from the one known for Begomoviruses and, to a lesser extent, the Mastreviruses.

We have used mutational analysis to identify firstly, the motifs in the BBTV Rep protein that are essential for replication initiation and secondly interfere with replication of BBTV. Specific mutations in motifs II and III completely destroy the ability of the Rep protein to initiate replication whereas mutations in motif I drastically reduce the rate of replication. Mutated motif I Rep however does not significantly interfere with the replication of BBTV when over-expressed in transient assays. Conversely, mutated motif II and III Reps greatly reduce the replication rate of BBTV in similar assays. This suggests that the mutated motif I Rep has lost the ability to bind to the BBTV intergenic region whereas mutated motif II and III Reps have retained the ability to bind but cannot initiate replication.

We have identified three potential iterons sequences within the BBTV intergenic regions. These iterons are highly conserved between the six ssDNA components of BBTV as well as being conserved between all characterized isolates of BBTV. The organisation of these iterons, with two forward iterons, F_1 and F_2, 3' of the stem-loop and one reverse iterons, R, 5' of the stem-loop, is very different to the organisation of the putative iterons of the members of the nanovirus genus and the members of the *Geminiviridae*. We have now demonstrated through mutational analysis that the iterons of BBTV have an effect on the efficiency of replication of this virus. Interestingly, alteration of the F_1 or R iterons had a significant effect on replication in contrast to alteration of the F_2 iteron, which resulted in the virtual abolition of replication.

These results have now provided us with information for the rational design of potential BBTV resistance genes based on transdominant negative resistance and Rep activated cell death.

Molecular analysis of banana streak virus integrants in the nuclear genome of *Musa balbisiana*

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Recently, it was demonstrated that banana streak virus (BSV) sequences were integrated into the nuclear genome of *Musa balbisiana.* There is strong experimental evidence that some of these BSV integrated sequences, called BSV endogenous pararetroviruses (BSV EPRVs), can give rise to infectious episomal BSV genomes under stress conditions, including interspecific genetic crosses. This phenomenon represents a serious limitation to the creation of new *Musa* hydrides that contain A and B genomes and to the diffusion of such hybrids.

As part of an international effort within the *Musa* Genomics Consortium, the integration patterns of BSV EPRV sequences in the nuclear genome and the mechanisms leading to the activation of these sequences are being identified. We have constructed and characterized three BAC libraries from the nuclear genomes of *Musa acuminata* 'Grande naine' (AAA), *M. acuminata* 'Calcutta 4' (AA) and *M. balbisiana* PKW (BB).

The complete genomes of four different BSV strains (BSV-Ol 'Obino l'ewai', BSV-Gf 'Gold finger', BSV-Im 'Imove', BSV-Mys 'Mysore') were used as probes to screen the BAC libraries and FISH experiments were carried out to test for the presence of integrated viral sequences in *Musa* A and B chromosomes. Upon screening the *M. balbisiana* PKW BAC library, we identified 10 BAC clones positive for BSV-Ol, 9 BAC clones positive for BSV-Gf and 24 BAC clones positive for BSV-Im. Screening of the 'Calcutta4' and Cavendish BAC libraries with the four complete viral sequences revealed that none of the BAC clones contained any BSV sequences.

BAC clones found positive upon screening were further characterized by BAC-end sequencing and RFLP-fingerprinting and the BAC clones containing BSV-Ol or BSV-Gf EPRV sequences were completely sequenced. Detailed analysis of the nucleotide sequences and chromosomal organisation of BSV-Ol and BSV-Gf EPRV sequences in these BAC clones will be presented and discussed. This study represents the first step towards characterizing the mechanisms leading to the activation of BSV EPRV sequences in *Musa* and the implementation of novel strategies to counteract this phenomenon.

Resistance to banana bunchy top nanovirus infection by Rep-activated suicide gene expression

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Banana bunchy top nanovirus (BBTV) is a single-stranded DNA virus that infects banana. We have developed a novel strategy for engineering resistance to BBTV in banana. The INPACT (in plant activation) system relies on an integrated, split suicide gene expression cassette that is flanked by the intergenic regions of BBTV. Suicide gene expression should only be activated in the presence of BBTV Rep, due to a replicational release mechanism. It results in cell death at the site of virus replication and ultimately virus containment. We have demonstrated the system is viable using transient assays with the β-glucuronidase (uidA) reporter gene, and are currently testing the system in stable transformants. The INPACT vector design for suicide gene expression may be applicable to all viruses that rely on a rolling circle mechanism of replication and therefore, may be of particular benefit to engineering resistance to other viruses including the Geminiviruses.

Isolation and analysis of differentially expressed mRNAs from activated banana streak virus integrated sequences

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The banana streak badnavirus (BSV) is responsible for banana streak disease, an economically important and widespread viral disease of *Musa*[1]. The impact of this disease on yield and quality is significantly high[1]. It has been reported that a strong correlation exists between the integrated BSV sequence and viral particles expressed from virus-free interspecific hybrids placed under stress conditions such as cold and *in vitro* culture[2]. BSV infection can occur in new inter-specific hybrids resulting from crosses between virus-free *Musa acuminata* and *Musa balbisiana* accessions. It has been observed that these BSV infections result from the activation of particular full-length viral integrants rather than from epidemic infections[2]. In our studies on characterizing BSV activation mechanisms underlying BSV expression and its regulation and host factors involved in BSV activation, suppressive subtractive hybridisation (SSH) libraries were constructed using stressed *in vitro* plants of cv. 'Penkelon' (AAB).

Various stress factors like chemical agents, antibiotic (hygromycin), polyamine (spermidine), demethylating agent (5-Aza cytidine) and heat shock (60^{o}C for 1 h) were tested. The episomal form of BSV expression in stressed plants was checked by PCR[3]. It was observed that BSV activation was very random and only 2-4 % of the plants showed BSV activation after 72 hours of stress in chemical, antibiotic and demethylating agent treated plants. Forward and reverse SSH libraries were constructed by isolating the total RNA from the BSV activated and BSV non-activated plants after being subjected to a stress for 72 hours.

Four SSH libraries with about 1500-2000 cDNA clones each have been constructed. These SSH libraries will be used for the isolation of host factors involved in BSV activation. Expression analysis of these cDNA clones using macro-arrays by reverse northern and virtual reverse northern for the isolation of candidate genes involved in BSV activation is in progress.

1. Lockhart B. and D.R. Jones. 2000. Banana streak Pp; 263-274 *in* Diseases of banana, abaca and ensete (D.R. Jones, ed.) CABI Publishing.

2. Dallot S., P. Acuna, C. Rivera, F. Cote, B. Lockhart and M.L. Caruana. 2001. Evidence that the proliferation stage of micropropagation procedure is determinant in the expression of banana streak virus integrated into the genome of the FHIA-21 hybrids (*Musa* AAAB). Arch. Virol. 146(11):2179-90.

3. Delanoy M., M. Salmon, J. Kummert, E. Frison and P. Lepoivre. 2003. Development of Real-time PCR for the rapid detection of Episomal Banana streak virus (BSV). Plant disease 87(1):33-38.

Molecular variability of banana mild mosaic virus

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Banana mild mosaic virus (BanMMV), a member of the Flexiviridae family, is currently one of the major viral constraints for exchanging *Musa* germplasm, since the virus is propagated vegetatively. Current data show that silmutaneous infections with Banana streak virus (BSV) or Cucumber mosaic virus (CMV) can lead to severe necrosis, whereas an infection by BanMMV alone causes no or only very mild symptoms. Very little information is currently available on the epidemiology of BanMMV, which has no known vector. In order to gain insights into the biology of BanMMV, we have undertaken studies on its molecular variability.

An IC-RT-nested PCR-based detection method was developed and amplification products within the RdRp gene were cloned and sequenced for 69 distinct accessions originating from CIRAD's *Musa* collection in Guadeloupe. A total of 154 sequences were analysed. Sequence comparisons showed an exceptionally high molecular variability between sequences originating from distinct accessions, while intra-plant variability was generally low. In a significant number of cases however, very high intra-plant diversity was observed. In other cases, very closely related sequences were isolated from different accessions suggesting transmission of BanMMV between plants. These results were confirmed by sequence comparisons of the 3' end of BanMMV genomic RNA covering part of the CP gene and the 3' untranslated region, performed with 30 more sequences originating from a selection of 10 out of the 69 above mentioned accessions. No conclusive correlation between the geographical or genetic origin of the accessions could be established.

These results tend to indicate that BanMMV could be preferentially but not exclusively transmitted vegetatively. They show that BanMMV is subject to a very active viral evolution process within isolated phyla. They also provide compelling evidence for movement of BanMMV isolates between accessions. Possible correlations between the very high variability observed and plant defence mechanisms will be discussed, as well as consequences of this variability on the development of efficient molecular tools for the detection of BanMMV.

Molecular characterization of an unidentified *Flexivirus*

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Phylogenic studies on banana mild mosaic virus populations led to the characterization of a 230 nt sequence from a yet unidentified virus found in one of the 69 accessions analysed. No visible symptom was associated with the presence of this virus. 3' RACE experiments were performed on double-stranded RNAs purified from this accession, enabling the cloning and sequencing of about 2.3 kb of the 3' end region of the genome of this virus. Based on sequence comparisons, the analysis of the cloned sequence showed the presence of 5 open reading frames encoding an RNA dependent RNA polymerase, three proteins involved in virus movement and organised as a triple gene block (TGB1, TGB2 and TGB3) and a coat protein, respectively. The genomic RNA ends in a short 3' non-coding region followed by a polyA tail. Sequence comparisons and phylogenic analyses performed on both nucleotide and amino acid sequences led to the conclusion that this virus, tentatively named *Banana X virus*, seems to represent a new agent since it shows only distant relationships with viral sequences present in databanks. It probably belongs to a new genus within the *Flexiviridae* family and is very distantly related to members of other genera in this family such as the genus *Foveavirus*. Results from molecular work aimed at unravelling the expression strategy of this virus will also be presented and discussed.

Session 1

Genetic resources and improvement

Posters

P1 - *In vitro* gamma ray mutation induction in cv. 'Kepok'

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'Kepok' is one of the most important banana cultivars in Indonesia and is used as raw material in chip making. Its tolerance to drought makes it suitable for the dry areas that are abundant in Indonesia. Both the domestic and international demands for the processed products of this crop are increasing and exerting a pressure to increase production. Unfortunately, no high yielding variant of this cultivar is currently available. Moreover, the fruits are harvested 1.5 to 2 years after planting, resulting in a very tall plant (3.5 m). These characteristics restrict the use of this variety in commercial plantations. A dwarf high yielding variety that could be harvested early would be useful. The objective of the research was to develop a dwarf (under 2.4 m) early flowering clone.

Mutations were induced in somatic embryo explants with dosages of 5, 6, 7, 8, 9 and 10 krad. The first selection was done based on visual observation of two-month-old plants and 750 plants, out of 5870, were selected. The selected plants were then treated with 100 mg/L of GA_3. The second selection of 29 plants was based on plant height, the length to width ratio of leaves and the distance between two successive leaves. The agronomic performance of these plants was evaluated in the field after two production cycles.

Two mutants, G-141 and H-18, were early flowering (9 months), two others, H-92 and H-45, and erect leaves were found on H-93 and H-94. These clones were different from the control plants based on an isoenzyme analysis using esterase and peroxidase enzymes (Figure 1).

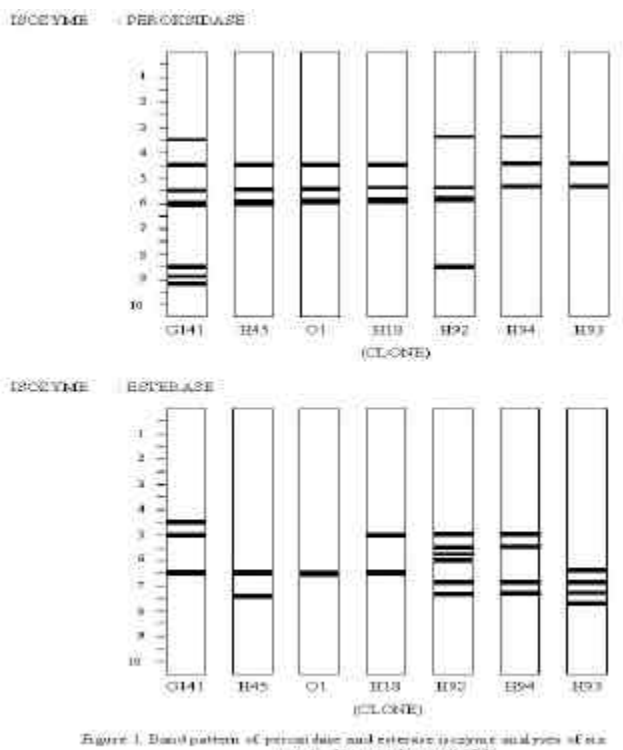

Figure 1. Band pattern of peroxidase and esterase isozyme analyses of two mutant clones and control (O1)

P2 - Employment of adventitious buds and gamma radiation in the induction of variability in ‘Grande naine’

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A protocol for plant regeneration via adventitious buds was developed for ‘Grande naine’. Several concentrations of BAP and TDZ in the culture medium were tested for their effect on the induction of these structures. The highest number of adventitious buds was obtained when TDZ was used in the culture medium, with only two sub-cultures needed. The explants derived from adventitious buds were then treated with 25 Gy of gamma radiation using ^{60}Co as the source and the phenotypic variations in the regenerated plants were evaluated and characterized. Six thousand plants were regenerated from irradiated adventitious buds and 5000 plants were regenerated from adventitious buds that had not been irradiated. In the population subjected to a mutagenic treatment, 16.6% of the plants showed phenotypic variability. In the population that was not irradiated, 6.6% of the plants showed phenotypic variability.

P3 - Somaclonal variation of tissue cultured explant of cv. 'Berangan'

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Random amplified polymorphic DNA (RAPD) was used to determine if the type of explant had any influence on the rate of somaclonal variation. A total of 45 DNA samples were extracted from male flowers, meristems and scalps of cv. 'Berangan' by using the CTAB method. Using six arbitrary 10-mer primers for amplification, they were analysed by PCR. A statistical analysis of the fragment patterns was carried out by using the Band Sharing and Similarity Index[1]. The similarity indices were 0.84 for the flowers, 0.81 for the meristems and 0.73 for the scalps. These results suggest that at the genomic level, there are more variation between plants derived from scalp explants, provided that all other factors were the same (e.g BAP concentration and the number of subcultures). Male flower explants appear more advantageous to use for propagation but a larger population should be evaluated and phenotypic observations recorded first. Another reason for using male flowers is the purported lower risk of viral contamination.

1. Odum E.P. 1971. Fundamentals of ecology. W.B. Saunders Company, Philadelphia.

P4 - Improvement of resistance to Fusarium wilt in ‘Manzano’ (AAB) and ‘Gros Michel’ (AAA) through tissue culture and mutagenesis

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The objective of this project was to select for somaclones resistant to race 1 and 2 of Fusarium wilt (caused by *Fusarium oxysporum* var. *cubense*) among irradiated plants of the susceptible ‘Manzano’ (AAB) and ‘Gros Michel’ (AAA) cultivars. Vitroplants regenerated from irradiated adventitious buds of both cultivars were inoculated with isolates of the fungus. After many cycles of selection in nurseries and in heavily infested fields, the selected clones were planted in field 32. These were evaluated over two production cycles and 9 ‘Gros Michel’ somaclones with an infection index inferior to 30% were selected. All the material derived from ‘Manzano’ was classified as susceptible after the first production cycle. The resistant somaclones that had the desirable agronomic characteristics were micropropagated *in vitro* and evaluated for their yield. Three somaclones with promising characteristics have been selecting.

P5 - Effect of chemical and physical mutagens on commercial cultivars

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Most bananas are highly sterile, seedless and triploid. These features are barriers for the implementation of conventional breeding. Mutagenesis coupled with improved micropropagation techniques is a logical and effective approach to improve qualitative characters in *Musa* species. Mutagenic studies using gamma rays as physical mutagens, and ethyle methyle sulphonate (EMS) and di-ethyle sulphate (dES) as chemical mutagens, were undertaken. Four economically important AAA cultivars, 'Robusta', 'Grande naine', 'Shrimanthi' and 'Basrai', were selected for studies.

The mutagenic treatments were given at various development stages using different concentrations of chemical mutagens (500 to 4000 ppm) and gamma rays (10 to 80 Gy). The treated cultures were subcultured and maintained over four cycles. About 2000 plants were successfully acclimatized and transferred to the field. Some promising results were observed under laboratory and field conditions.

All the cultures treated with more than 40 Gy died. Exposure to 20 Gy and low concentrations of both chemicals enhanced the multiplication rate. Higher concentrations of the chemical mutagens caused morphological aberrations. Early flowering was observed in 60% of the plants treated with low concentrations of dES. 'Robusta' plants treated with 10 and 20 Gy had a significantly higher bunch weight compared to control plants. Plants treated with high concentrations of both chemicals resulted in late flowering and abnormal fruit morphology such as curved fruits.

P6 - A method to establish embryogenic cell suspensions of 'Grande naine' by culturing male flowers in liquid medium

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The establishment of embryogenic cell suspensions using as initial explants immature male flowers of bananas cultivated directly in liquid medium has not yet been reported in the literature. In this study, immature male flowers, in positions 7 to 11 in relation to the floral apex, were used as initial explants. These were isolated and cultivated directly in M1 liquid medium[1] in 24-well plates (Falcon®). One explant was inoculated per well with 1.0 ml of culture medium and then placed on a rotary shaker at 100 rpm, total darkness and at a temperature of 27±2°C. The culture medium was changed every two weeks.

Disorganized growth was observed in the first two weeks, starting with an initial thickening followed by the appearance of yellow globules on the surface of the explants after five weeks of culture (Figure 1). The explants with the best results were from the ranges of 8, 9 and 10. Explants from position 11 showed good growth but a lot of blackening. The explants from position 7 were initially disorganized but did not form any yellow globules.

Some cells were observed through the inverted microscope after eight weeks of culture. The explants with the best results were subcultured in 25 ml Erlenmeyer flasks in which the cell suspensions were established after three weeks of culture in M2 medium[2]. At the beginning, the suspension comprised isolated meristematic cells, highly vacuolated cells, cell aggregates, pro-embryos and small yellow globules. After eight weeks in M2 medium, and subculturing every two weeks, type II and III cell aggregates predominated in the cell suspension[3] (Figure 2).

1. Escalant J.V., C. Teisson & F.X. Cote. 1994. Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivars (*Musa* spp.). Vitro Cell Dev. Biol 30:181-186.

2. Côte F, R. Domergue, S. Monmarson, J. Schwendiman, C. Teisson & J.V. Escalant. 1996. Embryogenic cell suspensions from male flower of *Musa* AAA cv. 'Grand Naine'. Physiol Plant 97:285-290.

3. Georget F., F.X. Côte, R. Domergue & N. Ferrière. 2000. Morphohistological study of the different constituents of a banana (*Musa* AAA, cv 'Grande Naine') embryogenic cell suspension. Plant Cell Reports 19:748-754.

Figure 1. *Yellow globular structures on floral buds after 5 weeks of culture.*

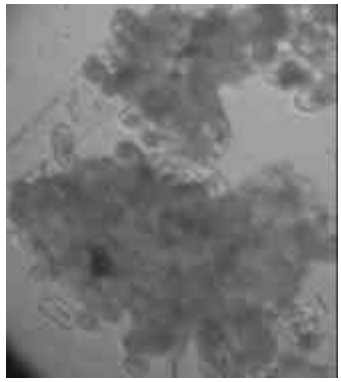

Figure 2. *Type III cell aggregates obtained after 16 weeks of culture.*

P7 - *In vitro* germination of *Musa balbisiana* embryos

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In the genus *Musa,* germination is highly variable and relatively difficult. Even more difficulties are faced when trying to produce hybrids. Authors[1,2] have reported on the limited and variable seed germination exhibited by *Musa. In vitro* embryo culture represents a potential tool for improving the recovery of hybrid germplasm in a short time.

In the present study, the *in vivo* and *in vitro* germination of two lines of *M. balbisiana* (10852 and MPL) seeds and mature embryos have been tested under various conditions. Seeds of both lines, sown in a peat-based mixture and on a wet filter paper or cotton in a Petri dish, were placed in the greenhouse routinely used to grow bananas. No *Musa* seeds had germinated after five months.

Sterilizing dry banana seeds is difficult. Even soaking them for more than a week in water did not improve sterilization and germination. Presterilization by soaking overnight in 0.16% sodium hypochlorite (NaOCl) helped sterilization. The seeds were then disinfected by immersion in 70% (v/v) alcohol for 8 min and mercuric chloride 0.1% (w/v) for 8 min, treated with NaOCl containing 1.6% active chlorine and 5% (v/v) ethanol supplemented with 0.01% (v/v) Tween 20 for 30 min, and rinsed 5 times with sterile water.

The seeds were cut longitudinally and the whitish mushroom-shaped embryos (Figure 1) were isolated. The excised embryos were inoculated on MS medium supplemented with plant growth regulators. The MS medium consisted of Murashige and Skoog salts and vitamins plus ascorbic acid (10 mg/l), sucrose (60 g/l) that was adjusted to pH 5.8, supplemented with 2.5 g/l Phytagel and autoclaved for 20 min at 121 °C. Ten and 25 ml of medium were dispensed aseptically to 50 and 100 mm disposable Petri dishes.

The following treatments were used:
MS: MS medium only
MSB: 2.2 µM BAP (6-benzylaminopurine)
MSD: 9 µM 2,4-D (2,4-dichlorophenoxyacetic acid)
MSG: 3 or 6 µM GA_3 (gibberellic acid)
MSP: 7.4 µM picloram.

Different temperatures (26°C and 29°C) and photoperiods (continuous light, continuous dark and 16/8 h light/dark) were tested. In 9 experiments, more than 2500 embryos were excised from seeds and inoculated on MS medium, with and without plant growth regulators.

After two weeks, 66% of the embryos kept in the dark on an MSB medium and 40% of those kept under a light/dark cycle on an MSP medium germinated. Lower germination frequencies were obtained with the other treatments and no germination was observed with the MS medium alone. Culturing at 29°C was not conducive to germination as all the germinations happened at 26°C. Germination was frequently higher in the dark than under a light/dark regime. In general, the growth rate and the shoot length of germinated plantlets were also better under dark conditions. The present study highlights the importance of various components for the *in vitro* culture of *Musa* embryos.

No germination was observed with the seeds or mature embryos of *M. balbisiana* MPL, regardless of the culture medium and conditions. The seeds may have been old or stored for a prolonged period under unsuitable conditions[1]. Selecting freshly harvested seeds appears to be important to obtain a higher germination frequency. In conclusion, in this study, the best conditions for embryo germination were darkness, 26°C and an MS medium with 2.2 µM BAP.

1. Simmonds N.W. 1952. The germination of banana seeds. Trop. Agric. 29:35-49.

2. Pancholi N., A. Wetten & P.D.S. Caligari. 1995. Germination of *Musa velutina* seeds: comparison of *in vivo* and *in vitro* systems. *In Vitro* Cell Dev. Biol. Plant 31:127-130.

***Figure 1**. A mature embryo of* Musa balbisiana 10852 *(left) and its germination on MS medium containing 2.2 µM BAP after 3 weeks (right).*

P8 - Regeneration of shoots from floral explants

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Immature inflorescences have been recognized as an important source of totipotent cells in many monocots and dicots. Inflorescence apices of banana have been successfully used for shoot production in many triploid clones [1,2,3]. Some of the wild and semi-wild diploid clones evaluated as potential sources of resistance to black leaf streak disease and Fusarium wilt exhibit low levels of suckering in the field and of proliferation in *in vitro* conditions. A trial was conducted to induce regeneration using floral explants.

'Kanaibansi' (AA), a potential source of resistance to Fusarium wilt and nematodes, was used. The bracts were removed from the male flower bud and partially mature flowers up to 5 cm in length and surface sterilized with 70% ethyl alcohol. Floral hands, ranging form 0.4 mm to 1.0 cm, were carefully isolated from the peduncle. The bracts of these floral hands were removed and inoculated in MS semisolid medium supplemented with different concentrations of 6 Benzyl Adenine (BA), 30g of sucrose and 100mg/L of myoinositol. The shoots regenerated from the floral explants were subcultured in MS semisolid medium supplemented with 3 mg/L of BA and multiplied. The shoots were elongated in MS medium and rooted in the same medium supplemented with 1 mg/L of IAA. All the cultures were kept under 16 hours of light and maintained at 25±2ºC. The rooted plants were transferred to soil.

The floral hands bulged and produced green globular mass like structures three weeks after inoculation (Figure 1). The production of similar structures was also recorded by Doreswamy and Sahijram[3]. The shoot buds were regenerated directly from this mass after 5 to 7 weeks of culture. The floral hands larger than 1 cm failed to produce shoot buds and simply developed into full flowers. Addition of BA was beneficial for the regeneration of shoots (Figure 2). A proportionate increase in regeneration with increased BA concentration was noted (Table 1). The highest number of shoots and percentage of response was recorded with 5 mg/L of BA, beyond which there was no beneficial response. The shoot clusters were further multiplied in MS medium supplemented with 3 mg/L of BA. The rooting of the regenerated shoots was facilitated with MS medium containing 1mg/L of IAA. The results suggest that the floral hands can be exploited for rapid multiplication of shy suckering *acuminata* diploids. This methodology is faster and comparable with a micropropagation protocol from floral apices. This is also an alternate technique for the production of multiple plantlets of selected clones after establishing their superiority through evaluation for multiple traits.

1. Bakry F., F. Lavarde-Guignard, L. Rossignol and Y. Demerly. 1985. Développement de pousses végétatives à partir de la culture *in vitro* d'explants inflorescentiels (*Musa* sp., Musacées). Fruits 40:459-465.

2. Cronauer S.S. and A.D. Krikorian. 1985. Reinitiation of vegetative growth from aseptically cultured terminal floral apex of banana. Am..J. Bot.72:1598-1601.

3. Doreswamy R. and L. Sahijram. 1989. Micropropagation of banana from male floral apices cultured *in vitro*. Scientia Horticulturae 40:181-188.

Table 1. Effect of growth regulator on shoot bud production from male floral hands (Data recorded 8 weeks after inoculation of floral hands; mean of 20 explants and experiments repeated five times).

Concentration of BA (mg/L)	Mean number of shoots per male flower	Percentage of response
1.0	1.6 e	12.4
2.0	2.4 d	22.7
3.0	4.4 c	36.4
4.0	5.2 b	58.8
5.0	7.2 a	76.6
6.0	7.0 a	76.0

Values followed by the same letter are not significantly different according to Duncan's Multiplication Range Test at 0.05 probability

Figure 1. *Production of cell mass.*

Figure 2. *Production of shoots.*

P9 - Effect of the post-thaw culture medium on the recovery of cryopreserved banana meristems

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The long-term conservation of banana germplasm has been initiated at the INIBAP Transit Centre, in Belgium, thanks to the development of cryopreservation protocols for banana meristems derived from *in vitro* cultures[1]. Amongst the various techniques that have been standardized, the most promising one is a fast-freeze/fast thaw method. In the present study, the effect of the post-thaw culture medium on shoot regeneration was investigated.

The proliferating shoot meristems of two *Musa* cultivars, namely 'Pisang serun' (*Musa acuminata*) and 'Robusta' (AAA), were generated[2]. White meristematic clumps of about 4 mm in diameter and containing at least 5 meristematic domes were excised and transferred to a preculture medium consisting of Murashige and Skoog's salts supplemented with 10 µM benzyladenine (BA), 1 µM indole-3-acetic acid (IAA), 0.4 M sucrose and gelled with 2.5% gelrite. These cultures were kept for 2 weeks under normal conditions for shoot growth. The sucrose-pretreated meristematic clumps were excised to obtain explants of 1-1.5 mm in diameter and containing 3-4 meristematic domes, which were subjected to a loading solution (2 M glycerol and 0.4 M sucrose dissolved in MS medium) for 20 minutes at room temperature. An ice cold plant vitrification solution 2 (PVS2) was added and kept for 150 minutes at 0°C. The PVS2 solution consisted of 30% glycerol, 15% ethylene glycol, 15% dimethylsulphoxide and 0.4 M sucrose dissolved in MS medium. Four to six clumps of meristems were placed in a droplet of the PVS2 kept on an aluminium foil strip (25 x 5 mm) and plunged in liquid nitrogen (LN). After 1 hour of storage, the meristems were thawed by immersing the aluminium foil in the deloading solution (1.2 M sucrose dissolved in MS medium). Three post-thaw regeneration protocols were tested:

1. The meristems were placed on 2 sterile filter papers on top of a semi-solid hormone-free MS medium containing 0.3 M sucrose. After two days, the meristems were transferred to a semi-solid regeneration medium (MS + 2.22 µM BA + 0.09 M sucrose, gelled with 2.5% gelrite) without filter papers.
2. The meristems were placed in liquid MS + 2.22 µM BA + 0.09 M sucrose medium for one week and transferred to a semi-solid regeneration medium.
3. The meristems were placed on 2 sterile filter papers on top of a semi-solid hormone-free MS medium containing 0.3 M sucrose. After two days, the meristems were transferred to a liquid regeneration medium (MS + 2.22 µM BA + 0.09 M sucrose) and a week later to a semi-solid regeneration medium.

The meristems exhibited two types of responses: regrowth/shoot regeneration and callus formation. Calli did not grow further due to necrosis. On average, shoot regeneration of unfrozen meristems using protocols 1, 2 and 3 was, respectively, 50±16.7%, 44.4±9.6% and 38.0±20.8% with 'Pisang serun', and 91.7±4.4%, 77.8±19.2% and 72.8±10.6% with 'Robusta' (Figure 1). For a given cultivar, the difference between the three protocols was not significant.

On average, post-thaw shoot regeneration of cryopreserved meristems using protocols 1, 2 and 3 was, respectively, 23.9±12.1%, 15.9±1.4% and 62.5±4.2% with 'Pisang serun', and 62.5±9.9%, 41.9±4.4% and 88.6±10.3% with 'Robusta' (Figure 1). Post-thaw survival was significantly higher using protocol 3, regardless of the cultivar. In a previous study[3], in which semi-solid medium and liquid medium with 0.1 and 0.2 M sucrose were tested on the cryopreserved meristems from 3 banana cultivars, the liquid medium with 0.2 M sucrose gave the best results. This increased response can be attributed to the dilution in the liquid medium of excreted polyphenols from the explants, which did not form an impermeable shield around the thawed tissues that would have prevented nutrient uptake.

Instead of using a liquid medium, the present study shows that 2 days of culture on semi-solid medium with 0.3 M sucrose followed by incubation in liquid medium increased post-thaw shoot recovery. The high sucrose medium may have helped overcome the osmotic shock of the explants subjected to high sucrose

(0.4 M) in the vitrification procedure and the regeneration medium (0.09 M). Further testing of this post-thaw protocol across several genotypes should reveal its applicability for cryogenebanks.

1. Panis B & N.T. Thinh. 2001. Cryopreservation of *Musa* germplasm. (JV Escalant & S Sharrock, eds). INIBAP Technical Guidelines 5. INIBAP, Montpellier, France, 44pp.

2. Panis B, H. Strosse, S. Van Den Hende & R. Swennen. 2002. CryoLetters 23:375-384.

3. Surga J.G., R. Swennen & B. Panis. 2001. Info*Musa* 8:23-24.

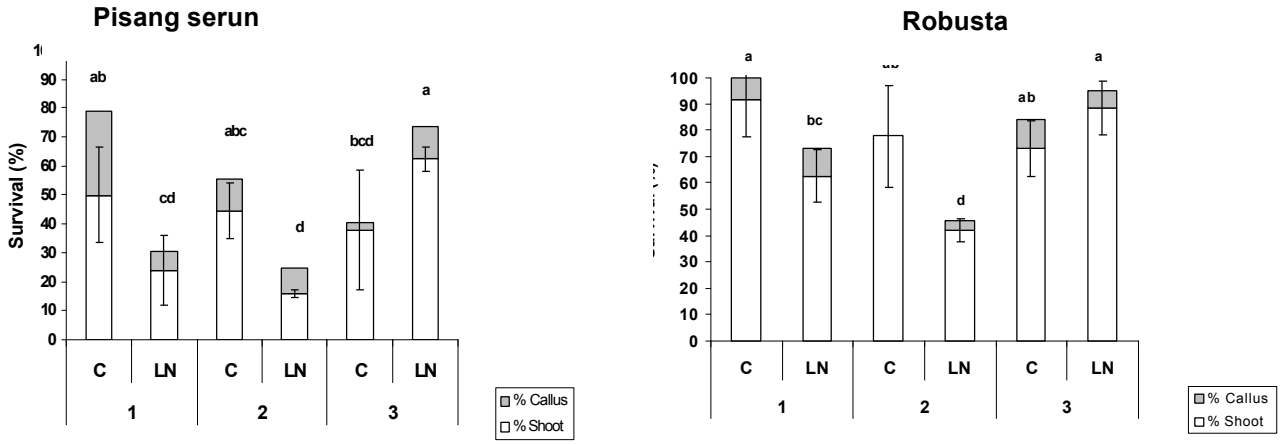

Figure 1. *Shoot regeneration and callus formation in banana cvs. 'Pisang serun' (*Musa acuminata*) and 'Robusta' (AAA). (Averages of about 10 explants/treatment in three replicates. Bars represent standard deviations. Different letters mean a significant difference according to Duncan's Multiple Range Test at p<0.05.)*
C: control
LN: after cryopreservation.

P10 - Protocols for establishing embryogenic cell suspensions and plant regeneration for gene transformation

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Protocols for the establishment of embryogenic cell suspensions have been developed. Immature male flowers from 'Mas' (AA) were used as explants. After three months of culture meristematic globules, and to a lesser extent calli, were induced to form. Yellow and friable embryogenic calli formed after 2 to 3 months of subculture on the same medium. Calli formed on 29% of the explants. To establish the cell culture, the embryogenic callus was suspended in liquid medium contained MS basal medium supplement with 2,4-D, biotin, glutamine and maltose at 100 rpm on a rotary shaker for one month (the medium was replaced every two weeks). The cultures were harvested and transferred to a somatic embryo induction medium. After 60 days the somatic embryos were transferred to a maturation medium for about 30 days. Germination increased from 18% to 39% when BA was replaced by thidiazuron.

We also investigated the factors affecting the first phases of an A*grobacterium*-mediated transformation system by detecting the transient expression of GUS gene controlled by CaMV35S or rice actin1 promoter in expression vectors constructed from pBA002. The results indicated that EHA105 was a more efficient strain than LBA4404. The transient expression rate of the GUS gene was five times higher when the explants were pretreated with 0.2 mol/L of mannitol. Five transgenic plants of cv. 'Brazil' and seven of 'Pisang awak' were obtained. PCR, PCR-southern blot and southern blot analysis have confirmed that the foreign gene had integrated the banana genome.

In a particle bombardment transformation system, we used the vector pTEW2402 containing antisense ACC oxidase gene (PTOM 13) with the reporter GUS gene to optimize the physical and biological conditions. Our results showed that the best pressure and the best distance to the target tissue were 1100Psi and 9cm, respectively. An osmotic treatment with 0.2 M of mannitol increased by nearly four times the transient expression rate when the treatment was done 4h before and 16h after the bombardment. Pre-culture of the explants on a regeneration medium also increased the expression rate. Eight regenerated plants were obtained, but only three had integrated the foreign gene.

P11 - Regeneration of *Musa* spp. from male inflorescences

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The use as explant of male inflorescences instead of meristems in the mass propagation of *Musa* spp. should reduce the risk of viral contamination. The cultivars 'Mas' (AA), 'Jari buaya' (AA) and 'Berangan' (AAA) and the subspecies *Musa acuminata* m*alaccensis* (AA) were tested. Three lengths of male inflorescences (1, 2 and 3 cm) and grown on MS initiation media with three concentrations of BAP (4.4, 31 and 70 µM). After three to five days, the explants turned greenish at the tip. The colour later spread to the base. More than 80% of the 2 cm explants and 90% of the 3 cm ones turned green whereas most of the 1 cm explants suffered necrosis. Within two weeks of culture, the bracts opened up to expose the immature male flowers and differentiation occurred to produce whitish bud-like structures in less than 2 months. The best growth of such structures was obtained with the media supplemented with 70µM of BAP. After 3 months, shoot-like structures emerged. The mean number of these structures obtained with media supplemented with 31 µM and 70 µM of BAP was not significantly different. Normal looking plantlets of *Musa* were obtained within 4 to 6 months depending on the variety.

P12 - Somatic embryogenesis from cell suspensions of cv. 'Rastali'

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Cell suspensions of a local dessert banana cv. 'Rastali' were established using immature male flowers. Callus was induced using different concentrations of 2,4-D. The highest percentages of embryogenic callus formation were obtained with 2 mg/L 2,4-D. They were 54.7%, averaged over all the flower cluster positions, and 83.8% for position 8. Position 8 produced the highest percentage (48.4%) of embryogenic callus for all levels of 2,4-D tested.

The embryogenic calli were transferred to S1 and S2 liquid media for development of embryogenic cell suspension cultures. The cell suspension cultures were sieved and subcultured at 4-week intervals. After the third subculture, fine granules (embryogenic cell clusters) produced in the suspension cultures were transferred to a hormone-free solid MS maturation medium. Within 2 months of culture, the embryogenic cell clusters developed into whitish translucent globular somatic embryos. Plantlets were obtained upon transfer of the somatic embryos to germination medium.

P13 - Protocol for plant regeneration from embryogenic cell suspensions of cv. 'Mas'

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Plant regeneration from somatic embryos is the critical stage in banana somatic embryogenesis. The current protocols tend to result in poor embryo development and a low plant regeneration frequency[1,2,3,4,5,6]. The objective of this study was to develop a simple and reliable regeneration system for banana embryogenic cell cultures.

Embryogenic cell suspensions of 'Mas' were established[1]. Embryo development on liquid and solid Murashige and Skoog media without plant growth regulators was compared. Resin-based histological techniques were used to study the differentiation of the developing somatic embryos in the liquid and solid media. The differentiated embryos were transferred to a regeneration medium containing MS macronutrients and micronutrients supplemented with 1 mg/L of thiamine HCl, 1 mg/L of nicotinic acid, 10 mg/L pyridoxine, 100 mg/L of myo-inositol, 7 mg/L of 6-benzylaminopurine, 30.0 g/L of sucrose and 2.6 g/L of phytagel at pH 5.8.

More embryos, about 32 000 per ml of settled cells, were obtained with the liquid MS medium than with the solid medium. Histological observations revealed better defined cambial tissue in embryos cultured on the liquid medium. Most of the mature embryos germinated and regenerated into plants within four months. The liquid protocol may have eliminated exogenous auxins and facilitated the dispersion of phenolic and other by-products.

1. Novak F.J., R. Afza, M. Van Duren, M. Perea-Dallos, B.V. Confer and X. Tang. 1989. Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa* spp.). Bio/Technology 7:154-159.

2. Dhed'a D., F. Dumortier, B. Panis, D. Vuyleteke and E. De langhe. 1991. Plant regeneration in cell suspension cultures of the cooking banana cv. Bluggoe (*Musa* spp. AAB Group). Fruits 46:125-135.

3. Côte F.X., R. Domergue, S. Monmarson, J. Schwendiman, C. Teisson and J.V. Escalant. 1996. Embryogenic cell suspensions from the male flower of *Musa* AAA cv. Grand nain. Physiologia Plantarum 97:285-290.

4. Becker D.K., B. Dugdale, M.K. Smith, R.M. Harding and D.L. Dale. 2000. Genetic transformation of Cavendish banana (*Musa* spp. AAA group) cv. 'Grand Nain' via microprojectile bombardment. Plant Cell Reports 19:229-234.

5. Ganapathi T.R., N.S. Higgs, P.J. Balint-Kurti, C.J. Arntzen, G.D. May and J.M. Van Eck. 2001. *Agrobacterium*-mediated transformation of embryogenic cell suspension of the banana cultivar Rasthali (AAB). Plant Cell Reports 20:157-162.

6. Jalil M., N. Khalid and R.Y. Othman. 2003. Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA). Plant Cell, Tissue and Organ Culture 75:209-214.

P14 - Mass production of plantlets from scalps of cv. 'Tanduk' (AAB)

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Scalps, which are clumps of white, compact, cauliflower-like proliferating meristems derived from *in vitro* proliferating shoot tip were chosen as the starting material for the establishment of embryogenic cell cultures of 'Tanduk' (AAB). Scalps 0.5 cm × 0.5 cm were cultured on Murashige and Skoog semi-solid medium containing half-strength macro and iron salts and supplemented with modified vitamins, 5μM 2,4-D, 1μM zeatin, 30 g/L sucrose and 7 g/L agarose (M1 medium). Callus with nodular structure and proembryos were obtained after 3-4 months of culture on the solid medium. The calli were transferred to liquid medium of the same composition (M2 medium) and after several subcultures a homogenous embryogenic cell line was obtained. Development of somatic embryos was achieved on transfer of 1.0 ml aliquots of the embryogenic cell suspension onto hormone-free MS medium containing Morel's vitamins, 30 g/L sucrose and solidified with 2 g/L. gelrite (M3 medium). Somatic embryos germinated into plantlets upon transfer to MS medium supplemented with 0.5-1.5μM BAP (M4 medium). The frequency of plant recovery ranged from 33.2 to 52.6%.

P15 - Somatic embryogenesis in cultivars ‘Mas’, ‘Rastali’ and ‘Tanduk’

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Embryogenic calli were induced from immature male flowers in cvs. ‘Mas’ and ‘Rastali’, and from a scalp derived from a shoot tip in cv. ‘Tanduk’. Flower clusters from position 4 to 10 formed embryogenic calli after 6 months of culture. In ‘Mas’, position 9 produced the highest percentage of embryogenic callus (44.1%) while in ‘Rastali’ the highest percentage of embryogenic callus (48.4%) was obtained from position 8 for all levels of 2,4-D tested. The best callus induction medium for ‘Mas’ and ‘Rastali’ was an MS medium containing 2 mg/L 2,4-D and supplemented with 1 mg/L biotin, 1 mg/L NAA, 1 mg/L IAA, 30 g/L sucrose and 7 g/L agarose. The percentage of embryogenic calli was 53.0% for ‘Mas’ and 54.7% for ‘Rastali’ for all flower cluster positions assessed. ‘Mas’ embryogenic calli were transferred onto solid Dhed’a medium and produced somatic embryos 6 months later.

Cell suspensions were established from ‘Rastali’ and ‘Tanduk’ embryogenic calli. The cell suspensions were subcultured at 4-week intervals. After 2-3 subcultures, fine granular embryogenic cell clusters were produced and transferred to a hormone-free solid MS maturation medium. After 2 months of culture, the embryogenic cell clusters developed into whitish translucent globular somatic embryos. Plantlets were obtained upon transfer of the somatic embryos to germination medium supplemented with 0.5-2.0 mg/L BAP.

P16 - Morphological study of wild banana seeds and embryos

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Seeds from wild *Musa* species are either barrel shaped and subglobose or angular shaped. However, detailed information on the seeds and embryos is lacking. As a consequence, a study on the morphology of the seeds and embryos of four *Musa* species namely *M. acuminata, M. gracilis, M. violascens* and *M. textiles* was undertaken. Mature unripe banana bunches were collected in Paroi (*M. acuminata*), Endau Rompin (*M. gracilis*), Sungai Buloh (*M. violascens*) and Indonesia (*M. textilis*). Seeds were extracted from ripened fruits and washed. The embryos were excised and viewed under stereomicroscope.

The seed of *M. acuminata* is either triangular or four-sided angular shape and is a dark brown. The seed is approximately 3 mm long by 6 mm wide. It is divided into two chambers, the larger portion containing the embryo and endosperm, and the smaller portion the chalazal mass. At the micropylar end of the seed, the inner and outer integuments form the characteristic micropylar collar. The micropylar plug consists of that part of the outer integument that surrounds the micropyle.

The seed morphology of *M. gracilis* and *M. violascens* are very similar. The seeds are long, cylindrical and divided into two regions, a blackish outer integument near the chalazal end and a brownish outer integument near the micropylar end. The latter occupies almost two thirds of the seed and is bulges slightly in the middle of the seed. The seed of *M. gracilis* has a rounded outline, almost barrel-like and is about 8 mm long and 4 mm wide. The surface of the outer integument is rough, uneven, with randomly distributed miniature protuberances. On the contrary, the surface of the outer integument chalazal of *M. violascens* is smooth. Compared to *M. gracilis,* the seed of *M. violascens* is smaller, about 6 mm long and 2 mm wide, and the outer integument tapers slightly towars the micropylar end.

The seed of *M. textilis* is very different. It is shaped like a berry and is the smallest seed among the four species, approximately 4 mm in diameter. Its surface is smooth and shiny black. In general, the anatomy of *M. textilis* seed resembles the one of the *M. acuminata* seed. The chalazal region constitutes approximately one tenth of the total volume of the seed. Despite variations in seed structure, all species had similar embryos, which were 1 mm long. The embryo is located under the micropylar plug, away from the chalazal end and surrounded by a powdery white endosperm. The yellowish white embryo resembles a button mushroom. It consists of an undifferentiated stalk-like epicotyl and a spherical radicle.

P17 - Effect of desiccation on the germination of seeds and embryos of *Musa acuminata* ssp. *malaccensis*

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Wild species of bananas are known to produce seeds, but their germination is erratic and unpredictable. This study looks at the germination potential of *Musa acuminata* ssp malaccensis.

Mature fruits were collected from the Serdang area and seeds were extracted from ripened fruits by prolonged washing. Surface-dried seeds were desiccated in an air-conditioned room (22^0C and 67% RH). Fresh seeds were desiccated to various target moistures and sampled for moisture determination and germination tests in sterile sand. After desiccation, the embryos were rescued, their moisture content determined and the embryos evaluated after culture in an enriched MS medium.

The moisture content of the fresh seeds and embryos was 42.9% and 40.4%, respectively. Desiccation decreased seed moisture content, with the embryo consistently having higher values compared to the seed. The trend in moisture loss can be divided into three phases: rapid loss in moisture during the first 20 hours, a slower rate for the following 30 hours and a very slow rate thereafter. Desiccation below 10% could only be achieved by using silica gel. The lowest moisture content obtained was 5.5%. Although the moisture content of seeds was low, the embryo excised from these seeds had a moisture content of 17%.

Germination of fresh and desiccated seeds occurred during the first 3 months, with considerable variations between the replicates. Attempts to break dormancy with chemical and hormone solutions, such as $GA_{3,}$ ethrel, ethanol and acetone did not improve germination. The highest percentage of germination was 40%. Due to the erratic nature of seed germination, a tetrazolium test was carried out to assess viability after desiccation. Interestingly, all levels of desiccation showed high viability.

In order to confirm the results of the viability test, embryo from the seeds were rescued after each desiccation treatment and cultured *in vitro*. High viability (90 to 100%) of the embryos in culture was observed, with healthy plantlet development within three weeks. Desiccation to below 15% reduced survival when embryos were excised directly from the desiccated seeds. However, when seeds were presoaked on wet filter paper for 16 hours prior to the excision of the embryos, a marked improvement in survival was observed. Under a 5% moisture level, a drastic decline in viability was observed. Based on these results, it is possible to store seeds with a 10% moisture content, especially if the embryo rescue technique is used for regeneration. Further studies on sensitivity to temperature are on going.

P18 - Effect of polyploidizing agents on cvs 'Matti' (AA) and 'Kunnan' (AB)

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Breeding for resistance to Fusarium wilt and black leaf streak disease are the major goals of breeding programmes. Although *Musa acuminata* ssp. b*urmannicoides* is currently used as the source for resistance to black leaf streak, the search is on for alternate gene sources. In the Indian breeding programme, cultivated and semi-wild diploids, such as 'Matti' and 'Kunnan', are being used in the development of synthetic diploids and in triploidy breeding. But their usefulness is limited by male and/or female sterility. Restoration of fertility at the tetraploidy stage has been demonstrated in banana[1, 2]. Trials were conducted to evaluate the response of diploid cultivars to the induction of tetraploidy and the efficiency of polyploidizing agents.

In the first trial, 'Kunnan' and 'Matti' shoot tips were soaked in colchicine for 24 hours and in oryzalin for 72 hrs, respectively. In the second one, they were respectively cultured in an initiation medium in which colchicine and oryzalin had been incorporated. A MS medium fortified with 3.0 mg/L of BAP was used for initiation. The same medium with reduced level of BAP (2.0 mg/L) was used for monthly subculturing. After the third subculture, the explants were rooted on MS medium without growth regulator. Induction of polyploidy was verified.

No significant difference was observed between the concentrations of colchicine tested (Table 1) except for the number of days to bud initiation during the second and third subcultures. The number of days to bud initiation was lowest in the control and with 10 mM. With oryzalin, the results were significantly different (Table 2), except for the number of buds per explant during the first and second subcultures. As with colchicine, the 10 µM concentration gave similar results to the control.

The results show that colchicine and oryzalin can be used for chromosome doubling without inhibiting *in vitro* shoot regeneration. Soaking was found to be a suitable method for *in vitro* polyploidization. Incorporation in the medium resulted in browning and eventual mortality of shoot tips (>80%). Oryzalin was found to be better than colchicine, as it accelerated bud initiation and their multiplication.

1. Hamill S.D., M.K. Smith and W.A. Dodd. 1992. *In-vitro* induction of banana autotetraploids by Colchicine treatment of micropropagated diploids. Australian Journal of Botany 40:887-896

2. Van Duren M, R. Morpurgo, J. Dolezel and R. Afza. 1996. Induction and verification of autotetraploids in diploid banana (*Musa acuminata*) by *in-vitro* techniques. Euphytica 88:25-34.

Table 1. Effect of colchicine on *in vitro* shoot regeneration in cv. 'Kunnan'.

Treatment	Initial culture	First subculture		Second subculture		Third subculture	
	Number of days to greening	Number of days to bud initiation	Number of buds per explant	Number of days to bud initiation	Number of buds per explant	Number of days to bud initiation	Number of buds per explant
Control	10.0	13.4	3.0	5.6 b	4.4	8.00 c	6.60
2.5 mM	9.0	25.2	1.4	17.2 a	2.2	12.80 b	4.80
5.0 mM	8.2	17.6	2.0	20.0 a	3.2	18.00 a	6.00
7.5 mM	10.0	22.0	2.4	19.4 a	1.0	13.40 b	3.20
10.0 mM	8.0	17.2	3.4	8.0 b	4.2	9.00 c	4.80
CD*	NS	NS	NS	3.02	NS	1.90	NS

*CD: critical difference at 0.05 probability

Table 2. Effect of oryzalin on *in vitro* shoot regeneration in cv. 'Matti'.

Treatment	Initial culture	First subculture		Second subculture		Third subculture	
	Number of days to greening	Number of days to bud initiation	Number of buds per explant	Number of days to bud initiation	Number of buds per explant	Number of days to bud initiation	Number of buds per explant
Control	9.8 b	19.5 b	1.6	10.1 c	5.8	6.0	10.3 a
2.5 mM	9.8 b	20.0 b	1.6	8.0 d	4.8	6.7	10.8 a
5.0 mM	12.0 a	22.3 b	1.5	12.3 b	2.3	8.8	4.1 b
7.5 mM	13.0 a	29.5 a	1.8	14.3 a	4.0	8.6	3.3 b
*CD	1.4	2.2	NS	1.3	NS	NS	2.2

*CD: critical difference at 0.05 probability.

P19 - Domestication of 'Namwa' and 'Saba'

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Bananas are one of the oldest cultivated fruits. We explored polymorphism at plastidic and nuclear gene loci to determine the history of domestication of bananas, with emphasis on the southeast Asian BBA/BBB complex, 'Namwa' and 'Saba'. Four plastidic marker loci were developed; *rpl*16, *psa*A, *ndh*A, and *pet*A. Using PCR-RF-SSCP analysis, several chloroplast haplotypes were detected within each of the *Musa acuminata* and *Musa balbisiana* species. The polymorphisms within each species included single nucleotide substitutions, length variation at mononucleotide repeat sites, and insertion/deletions.

Using PCR primers matched to conserved regions in four enzyme coding nuclear gene families [isocitrate dehydrogenase (*Idh*), alcohol dehydrogenase (*Adh*), isopentenyl pyrophosphate isomerase (*Ipi*) and granule-bound starch synthase (*Gbss*)], the corresponding genes in *Musa* were amplified and sequenced. One locus corresponding to an alcohol dehydrogenase coding region (*Adh2*) and one locus corresponding to part of the *Gbss* gene have been studied in detail. At each of the two loci, the A and B-genome derived alleles were distinguished relatively easily and a wide diversity was observed at each locus. In the hybrid cultivars, the alleles derived from each of the contributing genomes were separated. Several alleles of the A and B genomes have been sequenced from cultivars and wild relatives. The relationships between these alleles and what they reveal about the domestication of southeast Asian BBA/BBB bananas are discussed.

P20 - Conservation of banana accessions in the Federated States of Micronesia

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The Federated States of Micronesia (FSM) is a small Pacific island nation comprised of four States and hundreds of islands spread between the Republic of Marshall Islands and the US Territory of Guam in Micronesia. The diversity of bananas in this small nation is high and 82 cultivars have been reported. Many plant species in the FSM, including bananas and other staple crops, are under threat because of the small island environment. Increased anthropogenic pressure, habitat degradation, climate change, and its impact on the island ecosystem, and a lack of organized cultivation of several varieties that are not favoured for local consumption or trade, are major reasons for the amplified level of threat. Therefore, a conservation program combining *in situ* and *in vitro* methods was initiated to conserve banana germplasm. Due to the location of the Micronesia Plant Propagation Research Center, the only *in vitro* culture facility in the FSM, Kosrae was selected to be the conservation site. Collection of accessions from Kosrae State and the establishment of *in situ* plots and *in vitro* accessions are in progress.

P21 - Interesting bananas of Malaysia

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Banana is a very versatile crop; nearly every part of the plant can be put to use. The easy to maintain wild *Musa acuminata* can be used in landscaping, as well as the ornamental *Musa coccinea, Musa velutina, Musa ornata* and *Musa sumatrana* and the cultivated 'Pisang seribu' (AAB) and 'Pisang lilin' (AA), to name a few. In certain areas, 'Pisang Ceylan' (AAB), is popular at weddings. The attractive red fruit of 'Pisang raja udang merah (AAA) is used as offering by the Chinese community while the pith has medicinal purposes. The roots of 'Pisang gala' (BB) also have medicinal properties while the leaves are often used as plates.

'Pisang abu siam' (ABBB) produces a bunch with or without the male bud. A bunch with a male bud points downward whereas one without a male bud has fruits pointing upwards. 'Pisang serendah' (Dwarf Cavendish, AAA), also known as 'Pisang kapal' is very short and makes an attractive garden plant. 'Pisang jari buaya' (AA), which means crocodile fingers because of the shape of its fruits, is a popular sweet banana. 'Pisang bakaran' or 'Karang' (AAB) ripens green, has a sweet and sour taste and is usually eaten boiled. 'Pisang tanduk' (Horn plantain, AAB) has very strong apical dominance whereby as many as 50-60 suckers can sprout when the plant is cut down. There are two types of 'Pisang lemak manis' (AA): the green stem 'Pisang lemak manis kelantan' and the red stem 'Pisang lemak manis trengganu', which is lighter, has smaller fruits but a pleasant creamy sweet taste. The fruit of 'Pisang mas sagura' (AA), also known as 'Pisang perak', looks very much like that of 'Pisang mas' but is very seedy. 'Pisang geraksa' (AAB) is a popular cooking variety on the east coast with small fruits and is usually fried.

P22 - Diversity of Indonesian germplasm from Jasinga, Bogor

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Twenty-nine accessions from Jasinga, Indonesia, were studied. Genomic evaluation using the scoring system of Simmonds and Shepherd showed that 6 belonged to the AA group, 7 to the AAA group, 11 to the AAB group, 4 to the ABB group and 1 to the BB group. One cultivar, 'Klutuk hitam' (BB), was found for the first time. Fifty-one morphological characters were used to characterize the cultivars. They showed great variations in the size, shape and colour of the pseudostem, leaves, male buds, bracts and fruits. Agronomical data are also presented but they have not been correlated with the life cycle, resistance to biotic and abiotic stresses and fruit quality. A cluster analysis using Euclidean distance and average linkage showed that the closest relationship was between 'Subang' and 'Bapan', while 'Tanduk' was the most distant. Principle component analysis showed that 23 morphological character variables belonged to the first principle component, while 12 others belonged to the second principle component. The first principle component can be used to classify bananas according to their genomic constitution, whereas the second principle component can be used to differentiate sub-group of plantains from the other cultivars.

P23 - Leaf characteristics of various *Musa* accessions

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The leaf provides the plant with the carbohydrates needed for growth and development. Differences in leaf characteristics may lead to varied growth within the same genus. The present investigation was carried out to study the leaf characteristics of 19 *Musa* accessions grown at the Horticultural Research Station in Mondouri. The accessions were 'Monthan', 'Tholumvan', 'Matti', 'Red banana', 'Peyyan', 'Rashkaddli', 'Vellai tholuvan', 'Palayan kottai', 'Nendran', 'Kanthali', 'Daya', 'Giant governor', 'Malbhog', 'Agniswar', 'Pantharaj', 'Kanchakala' (var. Kalicharan), 'Kanthali champa' and 'Kanchakala uttam'. The thickness of the leaf at the base was highest in 'Kanthali' but the middle and terminal portion of the leaf were thickest in 'Daya'. With respect to chlorophyll, 'Nendran' had the highest concentration of chlorophyll-a while the levels of chlorophyll-b content were highest in 'Monthan'. The number of stomata per unit of leaf area varied significantly between accessions. However, stomatal size did not significantly vary between accessions.

P24 - *Musa* genetic diversity in West Bengal

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In West Bengal, banana is the second most important fruit crop and occupies 21 000 ha. There is a large diversity of bananas, particularly ABB cooking bananas and BB/BBB seeded bananas all over the state, especially in the Himalayan foothills and the adjoining districts of Bhutan and Assam. Over the last few years, the rapid expansion of areas cultivated with Cavendish clones and 'Martaman' ('Rasthali', AAB) is threatening the existence of cultivars with low productivity. The conservation of banana accessions in a field genebank has been developed at the Horticultural Research Station at Mondouri State Agricultural University in Mohanpur. At present, 45 banana accessions are maintained at the research station with a minimum of ten plants for each accession and their morphotaxonomic characterization is in progress. Some of the accessions collected from various parts of the state are 'Agniswar' (AAA), 'Chini champa' (AB), 'Pantharaj' (ABB), 'Kanthali' (ABB), 'Kanthali champa' (ABB), 'Champa' (AAB), 'Daya' (AAB), 'Krishna kanthali' (ABB), 'Kanchkala' types 1 to 5 (ABB), 'Amritsagar' (AAA), 'Manua' (BBB), 'Athia' (BBB), 'Bichi kala' (BBB), 'Hill banana' (AAB), 'Martaman' (AAB), 'Malbhog' (AAB), 'Sabri' (AAB), 'Penchi kala' (ABB).

The morphotaxonomic characterization of some of the cooking bananas has revealed variation in plant stature and bunch characteristics. A promising dwarf cooking banana (Type-3) with a high harvest index (0.65) has been evaluated for commercial cultivation by local banana growers. Unique relationships have been revealed by using a euclidean distance model on 10 genotypes. In addition to plant and fruit characters, ripening and the cooking characteristics of the cooking cultivars were also assessed.

P25 - Results of a collecting mission in the sub-Himalayan mountain range of India

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India is the cradle of *Musa* diversity, along with other south and southeast Asian countries. In India, the Northeastern states, Western Ghats, Eastern Ghats and Andaman and Nicobar Islands are hot spots of *Musa* diversity. The diversity of the Eumusa and Rhodochlamys series has been reviewed[1]. The present report continues the exploration of the sub-Himalayan mountain ranges and documents the diversity of the Rhodochlamys series, namely intersectional hybrids and their mutants.

Recent exploration conducted by the authors in the sub-Himalayan mountain range, especially in Arunachal Pradesh, the areas including Tawang, West Kemeng, West Siang, East Siang, Lower Subansiri and Lohit districts.

Six Rhodochlamys species were recorded during this collecting mission and their geographical distribution defined. *Musa*logue[2] notes the occurrence of only four species and Hakkinen *et al*.[3] reported six species

Musa laterita with brick red male bud and rhizomatous roots was distributed in upper Assam, around Tezpur, Balupara and Darranga. *Musa velutina* has a deep lilac male bud and dehiscent fruits. It has two centres of distribution, the West Kemang district of western Arunachal Pradesh and the Lohit district located in the eastern part of Arunachal Pradesh.

Musa rosaceae has a delicate lilac male bud, unusually long petioles and is distributed from East Siang, at an elevation of 400 m, to the Kimin, Yajali, Yachuli, Ziro, Raaga and Daparijo areas spread over three districts.

Musa sanguinea, which has a deep red male bud, has a localized distribution in upper Assam.

Musa aurantiaca is an important *Musa* spp. with a distribution extending from West Kemeng in the west to Lohit district in the east. A long presence in Arunachal Pradesh and the extreme climatic conditions have led to the occurrence of mutants with variation in pigmentation on the peduncle and fruits. Authors also noticed a unique mutant of *M. velutina* in the Gami, Bame, Daporijo and Along areas. The mutant exhibited reddish pink male bracts and fruits. The orientation of the hands was parallel to the axis and well spaced out. The patchy distribution of the mutant suggests that it must have occurred over a long period of time and was perpetuated in favourable environments.

The authors also came across natural intersectional hybrids of Rhodochlamys and Eumusa that were characterized by a medium stature, bright orange male bud (like *Musa aurantiaca*), the almost erect inflorescence characteristic of Rhodochlamys and the robust peduncle and biseriate nature of the fruits characteristic of Eumusa. These natural hybrids were observed in the West Kemeng district and the West and East Siang districts, located almost 500 km from each other. These areas are predominantly occupied by *Musa balbisiana, Musa nagensium, Musa itinerans* and two other species whose identity needs confirmation. *Musa acuminata* is distinctly absent in Arunachal Pradesh leading to the assumption that the new collection could be a hybrid between *Musa aurantiaca* and any one of the above species, except *Musa acuminata.* Molecular characterization is underway.

The compatibility of Rhodochlamys and Eumusa is well established and documented[4] and the results of our exploration strengthen this view. The newly identified mutants and natural hybrids add to the current spectrum of *Musa* genetic diversity.

1. Singh H.P. and S. Uma. 2000. Genetic Improvement of Banana in India - An overview. Pp. 136-156 *in* Banana - Improvement, production and utilization (H.P. Singh and K.L.Chadha, eds), AIPUB, NRCB, Trichy.

2. Daniells J., C. Jenny, D. Karamura and K. Tomekpe. 2001. *Musa*logue: A catalogue of *Musa* germplasm. Diversity in the genus *Musa* (E.Arnaud and S. Sharrock, compil). INIBAP, Montpellier, France. 213pp.

3. Hakkinen M. and S. Sharrock. 2001. Diversity in the genus *Musa* – Focus on Rhodochlamys. Pp. 16-23 *in* Networking bananas and plantains: INIBAP Annual Report. INIBAP, Montpellier, France.

4. Shepherd K. 1999. Cytogenetics of the genus *Musa*. INIBAP, France 160pp.

P26 - Improving ‘Pisang raja’ (AAB): exploiting natural diversity

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Malaysia is the home of many varieties of bananas. This diversity opens up opportunities for breeders and farmers to select for higher yield, disease tolerance and fruit quality. ‘Pisang raja’ (meaning king banana) is a dual-purpose cultivar. It can be eaten as a dessert banana, as is popularly done by the Chinese community, or cooked, which is very popular among Malays. Its yield, however, is low compared to other cooking varieties such as ‘Pisang nangka’, ‘Pisang abu’ and ‘Pisang awak’. Time to flowering is also long, 9-10 months, compared to the dessert bananas ‘Pisang mas’ (6 months), ‘Pisang berangan’ (7 months) and Cavendish (6-7 months). Being tall is another negative feature and like all commercial bananas, ‘Pisang raja’ is also susceptible to Fusarium wilt.

‘Pisang raja’ accessions were collected throughout peninsular Malaysia in some 90 locations. From 1 to 3 mats were collected per location with 1-4 suckers per mat. Planting was carried out under irrigation at MARDI, Serdang, using standard cultural practices. Time to flowering, plant height and girth, bunch weight, fruit length and tolerance to Fusarium wilt were recorded. The average yield was 14-16 kg, time to shooting was 250-300 days and plant height was 250-300 cm.

Above average accessions were selected from this first round of evaluation. One accession, 51-B, from Raub, Pahang, had a bunch weight of 16 kg for the mother-plant crop that increased to 17 kg and 23 kg in the first and second ratoon crops, respectively. This accession was still free of Fusarium wilt after three production cycles. Plant height, however, had increased from 280 cm to 399 cm by the second ratoon crop. Time to first flowering was 232 days. Other potential accessions include ‘Pisang raja sanggang’ and ‘Pisang raja ulu dong’. The selected accessions were multiplied *in vitro* and further selection was made for higher yields, early fruiting and tolerance to Fusarium wilt.

P27 - Genetic improvement of the French plantain 'Nendran' (*Musa* AAB) through conventional breeding

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The French plantain cultivar 'Nendran' is an integral component of farming systems in the south Indian state of Kerala, where it is also the commercial variety. The major constraints to its cultivation are its susceptibility to black leaf streak disease, corm and pseudostem borers and nematodes. The necessity to prop the plants to protect from wind damage increase production costs. One solution to these problems is the development of resistant varieties. Genetic improvement of plantains, though limited by low levels of female fertility, has been attempted with success. Development of plantain hybrids resistant to black leaf streak disease based on triploid/diploid crosses has been achieved[1,2].

Work along similar lines in progress at the Banana Research Station Kannara since 2001 is presented. Ten morphotypes of 'Nendran' were tested for female fertility by hand pollination with the wild banana *Musa acuminata* ssp. *burmanicoides* known for its high resistance to black leaf streak disease. Seed set was recorded in seven clones. Some AA diploid cultivars also proved successful as pollen sources. The average seed set however was very low and ranged from less than one to twelve per bunch. Seeds germinated better under mist. So far, the progeny tested in the field closely resembles the female parent in bunch characteristics and has an average crop cycle. The hybrid progeny also exhibits resistance to black leaf streak disease.

1. Rowe P.R. and F.E. Rosales. 1993. Genetic improvement of bananas, plantains and cooking bananas in FHIA, Honduras. Pages 243-266 *in* Breeding banana and plantain for resistance to diseases and pests: proceedings of an international symposium organized by CIRAD-FLHOR in Montpellier, France, 7-9 September 1992 (J. Ganry, ed). Montpellier, France.

2. Vuylsteke D., R. Ortiz and R. Swennen R. 1993. Genetic improvement of plantains and bananas at IITA. INFO*MUSA* 2(1):10-12

P28 - Field perfo*r*mance of tissue culture ‘Red banana’ plants

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In India, the cultivar commonly known as ‘Red banana’[1] (AAA) is restricted to pockets of Kerala, Maharashtra and Tamil Nadu. The limited distribution of the cultivar may be due to the non-availability of clean and disease-free planting material, non-hygienic field practices and the prevalence of bacterial and viral infections in all growth stages of the plant[2,3]

Sword suckers of ‘Red banana’ were used as explants in *in vitro* studies. Shoot tips were inoculated on various combinations and concentrations of NAA and IAA and/or BA and kinetine. The shoots were rooted after three subcultures and the regenerated plants were transferred to fields representing the four ecotypes of the Kariavattom, Monkompu, Kaliyakkavila and Vellayani banana growing regions. The height, length and girth of the plants, and the number, length and breadth of the leaves were recorded at planting and 30, 90, 180 and 270 days after. The plants produced fruits after 9 months. The number of hands and fingers, length, girth and weight of the fingers, and bunch were recorded. The number of suckers at harvest was also recorded and the suckers transplanted for further evaluation.

The growth of the plants cultured with the highest concentrations of BA alone or in combinations with NAA was exponential. The plants cultured with BA showed the best field performance over three generations. In view of the interest in the cultivation of ‘Red banana’, considerable research has been devoted to improving the techniques used for its mass propagation. The hormones and their concentrations play a major role in the field performance of this cultivar.

1. Abraham A. 1976. An inventory of germplasm of plants of economic importance in South India. Dept of Botany, University of Kerala, Trivandrum. 276pp.

2. Stover R.H and N.W. Simmonds. 1987. Banana. 3rd ed., Longman Scientific and Technical. New York.

3. Stover R.H., W.R. Hewitt and S. Chirappa. 1977. Banana. (*Musa* spp.). Pp. 71-79 *in* Plant health and quarantine in the international transfer of genetic resources. CRC Press Boca Raton. Florida.

P29 - Evaluation of *Musa* hybrids in Côte d'Ivoire

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Tetraploid banana and plantain hybrids produced by CARBAP (CRBP-14, CRBP-39, CRBP-85, CRBP-100), FHIA (FHIA-01, SH-3640, FHIA-23) and IITA (PITA-3, PITA-14, PITA-17, TMBx-15108-6) were evaluated in southern Côte d'Ivoire. Experiments were conducted at Azaguié (4°05 W longitude, 5°30 N latitude) according to INIBAP protocols.

Undesirable characteristics, such as breakage of the petiole and detachment of the leaf sheaths, which reduce photosynthesis, and the presence of seeds, which depreciates the quality of the production, were observed in some hybrids. The detachment of the leaf sheaths strongly affected CRBP-85. The proximal and distal breakage of the petiole mainly affected heavily foliated genotypes such as FHIA-23 and PITA-14 and, to a lesser extent, PITA-3. This disorder was frequently observed during the dry season. The leaf blade of CRBP-39 was often covered in black spots probably caused by an as yet not identified imperfect fungus. The fruits of CRBP-39 showed symptoms of diamond leaf spot disease, which was more frequent during the rainy season. Seeds were found inside the fruits of the hybrids TMBx15108-6 and PITA-17. The possible consequences of these traits are discussed with respect to the cultivation and domestic uses of these hybrids.

P30 - The observation of a two-bunch banana plant

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The present communication presents what we think is the first reported observation of a banana plant producing two healthy flowers (Figure 1). It took nearly four and half month for the fruits to fill. The bunches were estimated to weigh around 20 kg. The plant came from the sucker of a tissue culture plant.

Suckers produced by this plant were collected. Shoot tips were excised and inoculated on MS medium supplemented with benzyladenine and kinetin, for the proliferation of shoots. Other explants such as floral apices, leaves, roots and peduncles were also inoculated on MS media, with various combinations of growth hormones.

The lower middle portion of peduncle has shown the formation of shoots on a MS medium supplemented with the growth hormones 2,4-D and kinetin. It is too early to determine the viability of the shoots, but a rosette of leaves has been observed without any callus formation. Fruit quality and a host of agronomic parameters will be evaluated.

***Figure 1**. A two-bunch banana plant.*

P99 - Establishment of a banana transformation facility in South Africa for engineering Fusarium wilt and banana weevil resistance

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South Africa has a well-established banana industry based entirely on Cavendish varieties. In recent years, however, banana production has declined significantly in some production areas due to damage caused by Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense* race 4) and the banana weevil borer (*Cosmopolitus sordidus*). Neither Fusarium wilt nor the banana weevil can be controlled by means of cultural, chemical or biological control strategies. For the sterile and seedless Cavendish banana, improvement strategies such as the introduction of selected and characterized genes appears to be a promising alternative. The objective of this project was to establish in South Africa a banana transformation facility in which Cavendish bananas can be genetically engineered for resistance to Fusarium wilt and banana weevils.

Several putative gene sequences linked to Fusarium wilt resistance in bananas have been isolated by subtractive hybridization, and potential gene sequences will be further characterized for possible introduction into susceptible banana plants[1]. Resistance genes such as cysteine proteinase inhibitors are also being genetically mutated to enhance activity against the banana weevil[2]. Concurrent with these, embryogenic banana cell suspensions are being prepared to carry out *Agrobacterium*-mediated transformation. In this respect, male buds are being isolated from immature male flowers of high-yielding Cavendish bananas and incubated on 2,4-D containing MA1 medium for callus production. Embryogenic cell suspensions for somatic embryo production will be produced from ideal callus. Molecular marker techniques will also be developed to monitor the banana transformation/regeneration process to ensure the true-to-typeness of the engineered banana material. A flow chart will be presented on current activities.

1. Van den Berg N., P.R.J. Birch, A. Viljoen, M.J. Wingfield, M. Visser and D.K. Berger. 2002. Suppression subtraction hybrization used to identify genes associated with resistance to *Fusarium oxysporum* f.sp. *cubense* in Cavendish bananas. 3rd International Symposium on Molecular and Cellular Biology of Bananas, Leuven, Belgium. 9-11 September 2002.

2. Kiggundu A., K. Kunert, A. Viljoen, M. Pillay and C.S. Gold. 2002. Designing proteinase inhibitors for banana weevil control. 3rd International Symposium on Molecular and Cellular Biology of Bananas, Leuven, Belgium. 9-11 September 2002.

P100 - *Agrobacterium*-mediated transformation of 'Rastali'

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'Rastali' (AAB) plants were genetically transformed by co–cultivating single meristem buds with a disarmed *Agrobacterium tumefaciens* strain LBA 4404 harbouring the vector pROKLa-EG containing β-1,3-endoglucanase and neomycin phosphotransferase II (*npt II*) genes under the control of the cauliflower mosaic virus (CaMV) 35S RNA promoter. Adventitious buds were formed and shoots were regenerated both on kanamycin and geneticin G-418 selection medium. Integration of the transgenes and stable genetic transformants were assessed by PCR amplification of 830-bp of β-1,3-endoglucanase and 900-bp of *nptII* genes in transgenic plants derived from geneticin G-418 selection. Genomic Southern blot hybridization confirmed the incorporation of the single β-1, 3-endoglucanase gene into banana genome. β-1, 3-endoglucanase enzyme activity was three times higher in transgenic plantlets than in untransformed plantlets. These results also indicate that using geneticin 50 mg/L G-418 as a selection agent, instead of 100 mg/L kanamycin, is more efficient and does not significantly inhibit the multiplication rate.

P101 - *Agrobacterium tumefaciens*-mediated transformation of Cavendish and 'Lady finger' embryogenic cell suspensions

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A protocol on centrifugation-assisted *Agrobacterium*-mediated transformation developed using cultivars from two economically important genomic groups (AAA and AAB) will be presented. This protocol resulted in between 25 to 65 plants/50 mg of settled cell volume of embryogenic suspension cells, depending upon the *Agrobacterium* strain used. It also gave rise to hundreds of morphologically normal transgenic plants.

The development of a highly efficient *Agrobacterium*-mediated transformation protocol for a recalcitrant species like banana, especially the Cavendish group (AAA) cultivars, required the identification and optimisation of the factors affecting T-DNA delivery and subsequent plant regeneration. We used as starting material the male flowers from two banana cultivars (Cavendish and 'Lady Finger') and *Agrobacterium* strains AGL1 and LBA4404, harbouring binary vectors carrying *hpt* (hygromycin phosphotransferase) and *uid*A (β-glucuronidase) or *npt*II (neomycin phosphotransferase) and a modified *gfp* (green fluorescent protein) gene in the T-DNA, to investigate and optimize T-DNA delivery and tissue culture variables.

Factors that produced significant differences in T-DNA delivery and regeneration included the quality of the suspension cultures, the pre-induction of *Agrobacterium*, the inoculation and co-cultivation conditions and media, and the presence of acetosyringone and Pluronic F68 in the co-cultivation media. One factor that led to a significant increase in transformation frequency was the introduction of a centrifugation step during co-cultivation. Post co-cultivation liquid media wash and a recovery step helped avoid *Agrobacterium* overgrowth on the filters supporting the cell suspensions. Marker-gene expression and molecular analysis showed that the transgenes had integrated stably in the banana genome. T-DNA:banana DNA boundary sequences were amplified and sequenced in order to study the integration profile.

P102 - Biolistic-mediated transformation of cv. 'Mas' with a transcription factor associated with early flowering

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Plants with an early flowering gene would have an advantage over plants that develop normally. The aim of this study was to develop a stable transformation system to introduce into banana plants the pSOC1 construct, which contains sequences encoding for a MADS-box transcription factor associated with early flowering in oil palm.

Embryogenic cell suspensions of 'Mas' were established[1] and immature embryos bombarded with the plasmid pCAMBIA1301 containing the *gusA* gene driven by the 35S promoter. The biolistic experiment was carried out on a Bio-Rad PDS-1000/He system. Preliminary bombardment experiments had indicated that a helium pressure of 1350 psi at a distance of 6 cm from the target resulted in the highest transformation frequency.

The expression of *gusA* gene was detected histochemically[2] and fluorometrically[3]. The integration of the *SOC1* sequences was analysed by using Real-Time PCR (Rotor Gene, Corbett Research) according to QuatiTect™ Probe PCR Handbook. The transgenic lines are maintained for further analysis under laboratory conditions. The effect of *SOC1* on developing banana plantlets is currently being studied.

1. Jalil M., N. Khalid and R.Y. Othman. 2003. Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA). Plant Cell, Tissue and Organ Culture 75:209-214.

2. Jefferson R.A., T.A. Kavanagh and M.W. Beran. 1987. Gus fusion: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. The Embo Journal 3:3901-3907.

3. Jefferson R. A. 1987. Assaying chimeric gene inplant: The *uid*A gene fusion system. Plant Mol. Biol. Rep. 5:387-405.

P103 - The use of antifungal proteins to engineer resistance to *Fusarium oxysporum*

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Fusarium wilt of banana, which is caused by *Fusarium oxysporum* f.sp. *cubense* (Foc), poses a potential threat to commercial plantations of bananas in Malaysia. Since tolerant cultivars for this disease are not available and chemical control is ineffective, an alternative control measure is urgently required. One possible approach is to genetically engineer fungal resistance by introducing foreign antifungal gene into the genome of important but susceptible banana cultivars. Antifungal proteins are naturally produced in plants as part of the metabolic cascades triggered in response to a pathogen. A number of plant proteins exhibiting antifungal activity against a variety of plant pathogens have been isolated. Such proteins possess a moderate but wide spectrum fungicidal activity, which in some instances is due to the enzymatic property of proteins destroying the physical integrity of the pathogen's cell wall. Homologue proteins from microorganisms are reported to have stronger fungicidal activity than their counterparts in plants and have become the preferred source of antifungal proteins for genetic manipulations.

We report on the progress in introducing the endochitinase gene (*Chi42*), an antifungal gene from a local isolate of *Tricoderma hazianum* UPM40, into the genome of selected banana cultivars. The gene was amplified from the genomic DNA of *T. hazianum* by using a standard PCR protocol with various combinations of gene-specific primer sequences specifically designed to facilitate Gateway-based cloning approach by introducing the *att*B1 and *att*B2 sites to the 5' end of the forward and reverse primers, respectively. The *att*B-flanked PCR fragments were inserted into the pDONR^{TM}221 vector to create entry clones. The cDNA corresponding to the gene was also amplified and cloned in the same way. Subsequently, the genes in the entry clones were transferred to the binary T-DNA destination vectors to make expression vectors. A series of expression vectors containing *Chi*42 under the controls of CaMV 35S (pMDCUPM401), PAL promoter (pMDCUPM402) and *Chi*42 promoter (pMDCUPM403) were made for various expression analyses. Functional analyses of the constructs were performed on *Arabidopsis* before being applied to bananas.

P104 - Isolation of plantain promoters using the firefly luciferase reporter gene

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An *Agrobacterium*-mediated transformation was performed on the cultivar 'Three hand planty' (AAB) to isolate plantain promoters. The *Agrobacterium* strain EHA105 carrying a T-DNA with a promoterless firefly luciferase reporter gene next to the left border was co-cultivated with embryogenic cells. Following a selection of two months, 2602 independent cell colonies were screened *in vitro* for Baseline Luciferase Activity (BLA) with a liquid nitrogen-cooled CCD camera coupled to an image analysis system. Positive lines showing luciferase activation were further screened at the plantlet stage. The proportion of lines with BLA activity comparable or higher than that of the CaMV35S promoter control was 0.15%. Of the three lines selected for further analysis, isolation of the flanking plant sequences was accomplished by TAIL PCR with an average of 2.3 sequences per line. Sequences were compared to databases and analysed for conserved regions and putative transcription factor binding sites. Four out of the 7 flanking sequences of the putative promoter tagged lines contained vector backbone integration or T-DNA rearrangements. Three putative promoter sequences were isolated and their characterization will be presented.

P105 - The genetic potential of the cultivar ‘Agung semeru’

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The Lumajang Regency in East Java, Indomesia, contains 33 cultivars of dessert and cooking bananas. One of the cooking bananas is the cultivar ‘Agung semeru’. This variety grows well between 450 to 650 m above sea level and produces 1 to 2 suckers per mat. The fingers are 33 to 36 cm long and 19 cm in circumference. There is only 1 to 2 hands per bunch. The fruits have a thick peel and can be kept between 3 to 4 weeks in storage after harvesting. The pulp is sweet and can be eaten even when the peel has turned black as the fruit does not become soft. This cultivar is also resistant to black leaf streak disease.

P106 - Comparison of *Musa balbisiana* accessions from the Indian mainland and the Andaman and Nicobar Islands

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The *Musa balbisiana* B genome is an important source of genes conferring resistance to various biotic and abiotic stresses. The B genome has ben little studied because of its limited diversity. In India, the northeastern States, Western Ghats and the Andaman and Nicobar Islands are reported to harbour diversity of *Musa balbisiana*[1].

Recent exploration conducted in the south and middle Andamans resulted in the collection of 13 wild types (BB) and their diversity was compared to the diversity on the mainland. A total of 29 *balbisiana* accessions were characterized using RAPD markers. The genetic diversity and phylogenetic relationships were analysed in relation to the geographical location.

In the present study, 100 random primers were evaluated, of which only two primers, OPA 11 and OPD 03, were retained. The phylogenetic tree obtained with the OPA 11 primer exhibited revealed two clusters based on geographical origin. Wild types from the Indian mainland clustered separately from those from the Andaman and Nicobar Islands (Figure 1). The similarity between the two clusters was less than 45%

Cluster I, representing the wild B genome from the mainland, was further divided into two sub-clusters that were dissimilar by almost 25%. 'Borkal baista', from Assam, and 'Bhimkol-1' and 'Manguthamng' stood separately in the cluster and the last two were found to be synonyms. The other clones had some degree of similarities irrespective of their origin and distribution.

The second cluster included all the clones from the Andaman and Nicobar Islands. Of the 13 clones, 'New wandoor' was found to be unique. Though many of the accessions were collected under the same name in the same geographical location, they showed certain degree of diversity.

The isolation of the islands from the continents and between the islands has facilitated the evolution of a distinct and diversified flora[2]. The occurrence of B rich genomes in the Nicobar Islands has been reported[3]. Systematic exploration in the northern Andaman and Nicobar Islands is expected to reveal more diversity.

1. Uma S., B. Shyam, R. Selvarajan and S. Sathiamoorthy. 2001. Exploration Programme in Northeastern India. Pp. 103-109 *in* Advancing Banana and Plantain R & D in Asia and Pacific (A.B. Molina and V. Roa, eds). INIBAP-ASPNET, Los Baños, Philippines

2. Rao P.S.N. 1996. Phytogeography of the Andaman and Nicobar Islands, India. Malayam Nature Journal 50:57-79.

3. Singh D.B., P.V. Sreekumar, T.V.R.S. Sharma and A.K. Bandyopadhyay. 1998. *Musa balbisiana* var. *andamanica* (Musaceae)- A new banana variety from Andaman Islands. Malayam Nature Journal 52(3-4):157-160.

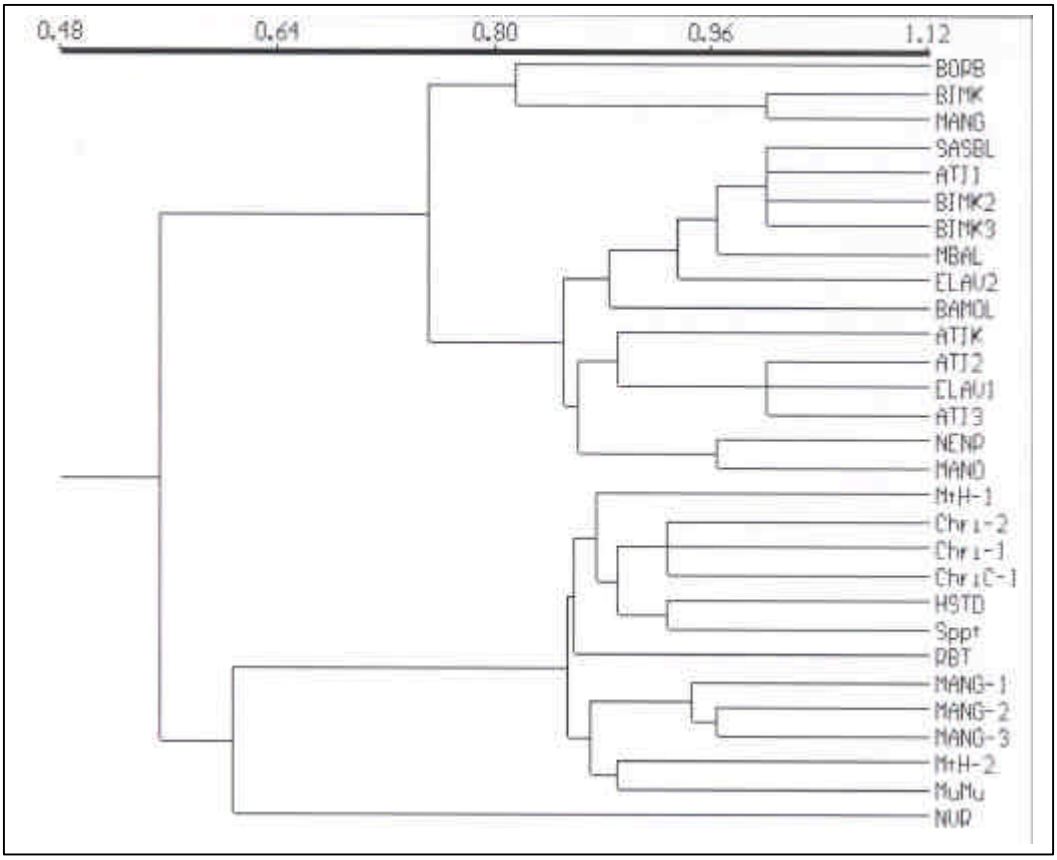

Figure 1. *UPGMA phenogramme comparing the B genomes from the Indian mainland and the Andaman and Nicobar Islands.*

P107 - Molecular characterization of *Musa* diploids using RAPD markers

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India is well known for the genetic diversity of its bananas and plantains, which include wild species and seedless cultivars of various ploidy levels. This is related to the fact that *Musa* originates from south and southest Asia, including the Indo-Burmese region. The south Indian states of Tamil Nadu, Kerala and Karnataka have a large diversity of edible diploids (AA and AB), some of which have not had their genetic group identified. The prevalence of many synonyms also creates problems for taxonomists. Morphological characteristics have proven very useful for clonal identification and taxonomical studies and morphology-based keys have been developed[1] (IBPGR 1998). Although taxonomic traits can reliably differentiate clones, the discriminating ability of this technique weakens as the genetic base of the clones under examination narrows[2].

The random amplified polymorphic DNA (RAPD) technique has been successfully used to distinguish *Musa* accessions[3]. In this study, 22 *Musa* diploids belonging to AA and AB groups were subjected to the technique using 25 primers. The accessions showed a high degree of polymorphism for primers OPF-4, OPF-8, OPF-9, OPF-12 and OPF-17. It was possible to distinguish all the cultivars by RAPD analysis. The results were in agreement with those of an earlier classification based on morphological characters. The cluster analysis and dendrogramme obtained with the RAPD data revealed distinct clusters of AA and AB groups. A large variation was detected among the diploids. An overall similarity of 63% was found among the diploid cultivars. Greater diversity was seen within the AA group than within the AB groups. Many natural mutants, such as ‘Ambala kadali’, ‘Erachi vazhai’, ‘Thattila kunnan’, ‘Vennetu kunnan’ and ‘Rasa kadali’ showed significant variation from their parental genotypes. The RAPD technique was useful in characterizing and discriminating between the diploids analysed. The breeding potential of these diploids will be discussed.

1. IPGRI. 1998. Descriptors for banana (*Musa* spp.). IPGRI, Rome, Italy.

2. Jarret R.L. and N. Gawel. 1995. Molecular markers, genetic diversity and systematics in *Musa*. Pp. 67-83 *in* Bananas and Plantains (S. Gowen, ed.). Chapman and Hall, London, UK.

3. Bhat K.V. and R.L. Jarret. 1995. Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. Genetic Resources and Crop Evaluation 42:107-118.

P108 - The use of RAPD markers to detect variability between AB cultivars

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Banana cultivars belonging to the AB genomic group, based on their morphological characteristics, are said to have originated in southern India. Commonly known as the Ney Poovan group, they form the most popular backyard cultivar in southern parts of India, especially in Karnataka and Kerala[1]. The fruits are liked for their firm pulp, acid sweet taste, thin peel and long shelf life. The cultivar 'Elakki bale', one of the important commercial cultivars of Karnataka, fetches high returns for the farmers. 'Kunnan' is another important backyard cultivar in Kerala state.

There are a number of reports on use of isoenzymes, RFLP, RAPD and AFLP markers to describe the phylogenetic relationships between banana cultivars and *Musa* species[2, 3, 4, 5, 6.]. An attempt was made to study the variability between the 17 AB cultivars available in our collections by using RAPD markers. Of the 80 primers that were screened, 16 showed polymorphism. The scorable polymorphic bands were analysed by using cluster analysis and principal component analysis (Figures 1 and 2). The results showed that there were two distinct clusters separating the Ney Poovan types and Kunnan types. Variability, attributed to somaclonal variation and natural mutations, was also observed in each group.

1. Bhakthavatsalu C.M. and S. Sathiamoorthy. 1979. Banana clonal situation in India. A resume. Fruits 34(2):99-105.

2. Gawel. S.J, R.L. Jarret and A.P. Whittemore. 1992. Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. Theoretical and Applied Genetics 84: 286-290.

3. Bhat K.V., S.R. Bhat and K.P.S. Chandel. 1992. Survey of isozyme polymorphism for clonal identification in *Musa* I Esterase, acid phosphatase and catalase. Journal of Horticultural Science 67:501-507.

4. Kaemmer D., D. Fischer, R.L. Jarret, F.C. Baurens, A. Grapin, D. Dambier, J.L. Noyer, C. Lanaud, G. Kahl and P.J.L. Lagoda. 1997. Molecular breeding in the genus *Musa*: a strong case for STMS marker technology. Euphytica 96:49-63.

5. Engelborghs.I, R.Swennen and S.V. Campenhout. 1996. The potential of AFLP to detect genetic differences and somaclonal variants in *Musa* spp. INFO*MUSA* 7(2):3-6.

6. Howell E.C., H.J. Newbury, R.L. Swennen, L.A. Whithers and B.V. Ford-Lloyd. 1994. The use of RAPD for identifying and classifying *Musa* germplasm. Genome 37:328-332.

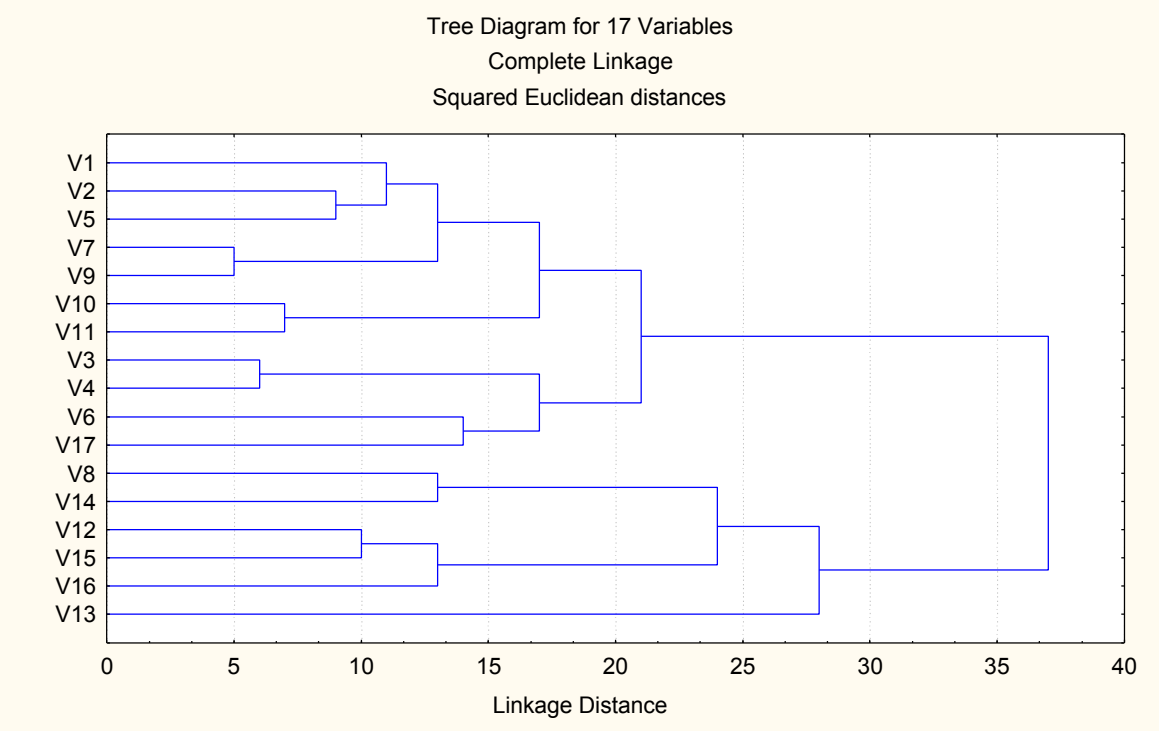

Figure 1. *Dendrogramme showing the relationships between the AB cultivars.*

V1: 'Hoo bale', V2: 'Ney poovan', V3: 'Njali poovan' (type 1), V4: 'Njali poovan' (type 2), V5: 'Safed velchi', V6: 'Putta bale' (type 1), V7: 'Putta bale' (type 2), V8: 'Batheesa paro', V9: 'Elakki bale', V10: 'Chakkara kadali', V11: 'Vaddakkan kadali', V12: 'Vannetu kunnan', V13: 'Valia kunnan', V14: 'Kunnan', V15: 'Adukka kunnan', V16: 'Chundilla poovan', V17: 'Mittili bale'

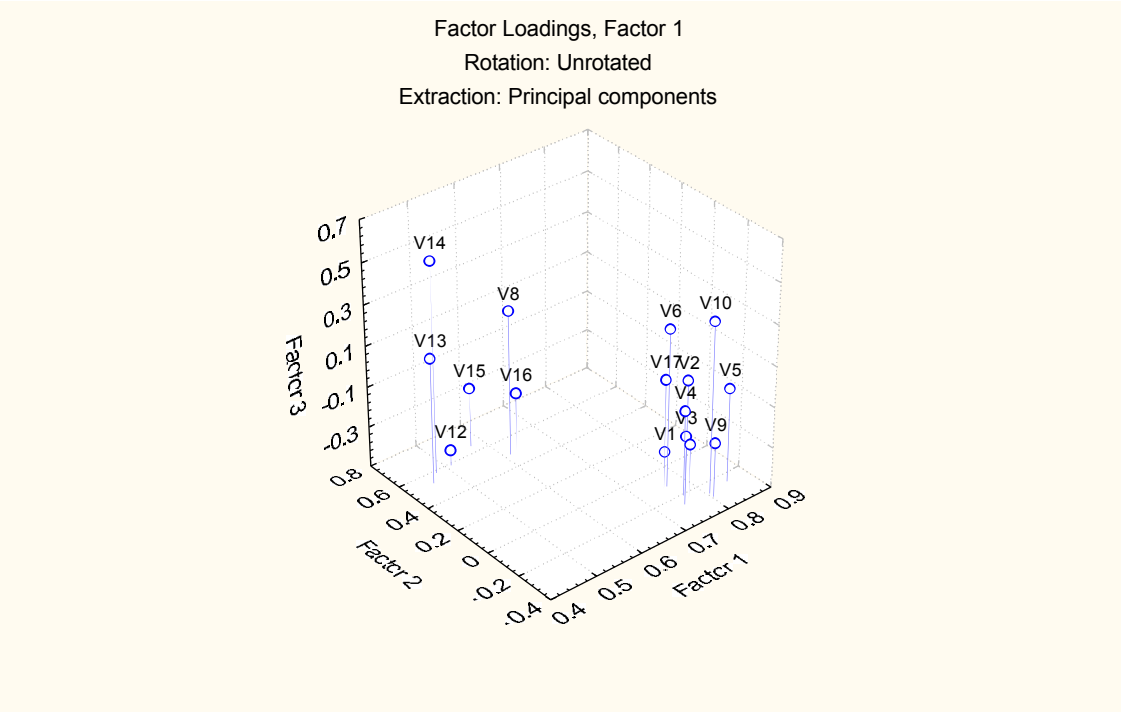

Figure 2. *PCA showing variability between AB cultivars.*

P109 - Application of a DNA marker system for assessing *Musa* germplasm

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Uncertainties associated with conventional characterization techniques impose limitations to the definition of diversity. More so when germplasm collections contain accessions that have different traditional names. Recent advances in molecular biology provide new tools to analyse diversity. DNA markers, in particular, can potentially analyse an unlimited number of loci distributed over the entire nuclear genome and as such detect variation within and among species[1]. Amongst the many systems available each has its advantages and disadvantages that have to be considered in ultimately deciding on a universal approach.

One approach that we are using to examine the diversity in the MARDI *Musa* germplasm collection relies on the use of repetitive DNA sequences in a simple reproducible PCR assay. The technique, Inter-Retrotransposon Amplified Polymorphisms (IRAP) essentially examines polymorphism in retrotransposon insertion sites between retrotransposons. The markers are co-dominant and show sufficiently high polymorphism to potentially be used to fingerprint the accessions. Ultimately a robust, reproducible fingerprint covering a reasonable number of loci may be the basis for defining a “banana barcode” for characterizing *Musa* germplasm.

1. Phillips R. and I.K. Vasil. 1994. DNA based markers in plants. Kluwer academic Publishers.

P110 - The use of DNA markers to differentiate variants of 'Rastali'

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'Rastali' (AAB) one of the most popular dessert banana cultivars in Malaysia is highly susceptible to *Fusarium oxysporum* f.sp. *cubense* (Foc) race 4. A somaclonal variant selected from tissue culture plantlets is showing tolerance to race 4. The diversity of alleles in 'Rastali' and its somaclonal variant were measured using microsatellites and Random Amplified Polymorphic DNA (RAPD) markers. The results revealed polymorphism within and between the variants. This study demonstrates the potential utility of this technique for discriminating between variants.

P111 - Use of random amplified microsatellite polymorphism for genetic characterization of local banana cultivars

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DNA markers have been showned to be a reliable tool in determining genetic variation and relationships between banana cultivars. The Random Amplified Microsatellite Polymorphism (RAMP) technique has been used in detecting DNA polymorphism in various organisms. In this study, RAMP was employed to determine genetic variation and the relationships between selected local banana cultivars. Eight cultivars were used for this study, namely 'Mas' (AA), 'Berangan' (AAA), 'Raja' (AAB), 'Rastali' (AAB), 'Awak' (ABB), 'Nipah' (BBB), 'Kapas' (AA) and 'Nangka' (AAB). Twenty-five individuals of each cultivar were collected from Perak, Selangor, Melaka and Negeri Sembilan. The DNA samples from leaves were extracted, quantified and purified. A total of five anchored-primers, KKVRVRV$(CT)_6$, GCTAGTGCT$(CA)_7$Y, GCACATGCAR$(TG)_7$, GATGCTGATR$(CA)_7$ and CATGCAT$(TG)_7$, were selected to amplify genomic DNA using the polymerase chain reaction (PCR). The banding patterns were observed and analyzed from the amplified DNA fragments.

A total of 147 scorable bands between 225 bp to 1700 bp were observed. The highest percentage of polymorphic bands was found in 'Kapas' (51.4 %) followed by 'Mas' (50.1 %), while the lowest was found in 'Nangka' (39.8 %). The highest genetic distance was found between 'Nangka' and 'Mas' (0.704), while the lowest was between 'Kapas' and 'Awak' (0.557). Based on the dendrogram, the eight banana cultivars were grouped into 2 clusters. The major cluster consisted of 'Berangan', 'Raja', 'Rastali', 'Nipah', 'Kapas', 'Awak' and 'Nangka', while the diploid 'Mas' cultivar was distinctly isolated from the major cluster. This study suggests that RAMP markers can be used to determine ploidy and evaluate the genetic variation between banana cultivars.

P112 - The application of *Musa acuminata* microsatellites in *Musa balbisiana*

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A set of *Musa acuminata* microsatellites were used to study the diversity between accessions of wild *Musa balbisiana* accessions from The Philippines and Thailand. Several of the microsatellite primers did not amplify fragments of wild *M. balbisiana* accessions. Those primers that did amplify a fragment, did not generally reveal polymorphismin in size length. Moreover, the amplified fragments were generally of a very different size, compared to *M. acuminata* accessions. Cloning and sequencing of sixteen alleles at one of the microsatellite loci (AGMI121-122) confirmed that the repeat was less polymorphic in *M. balbisiana* than in *M. acuminata*. Several base substitutions and insertion deletions were found in the sequence flanking the repeat. A phylogenetic analysis of the flanking sequences grouped the alleles in three clearly distinguished clusters: wild *M. balbisiana* alleles from Thailand, *M. acuminata* cultivar alleles, and some cultivars, such as 'Gobusik'.

Sequence analysis of one of the *Adh* loci (*Adh1*) revealed the presence of a highly polymorhic microsatellite in the fifth intron. The flanking sequences at this locus are highly conserved *M. acuminata* and *M. balbisiana* sequences, thus allowing amplification across both species (and also others). This locus can be used to assess diversity within both species, although the lengths of the amplified fragments overlap substantially. Microsatellite fragment length alone makes it difficult to make any inference about evolutionary relationships because of homoplasy (i.e. the independent evolution of similar fragment lengths).

P113 - New *Musa acuminata* microsatellite markers

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Bananas and plantains are the fourth most important food commodity after rice, wheat and maize. Yet, despite the importance of this crop in tropical regions, little attention has been given to its genetic improvement compared to the other major food crops. In an effort to develop freely accessible resources for *Musa* genomics, INIBAP launched the Global *Musa* Genomics Consortium in 2001. Microsatellite (or simple sequence repeat, SSR) markers have proven useful for genetic analysis in a number of species, including *Musa*. A large number of microsatellite markers would benefit banana breeding programmes, particularly for germplasm characterization, marker assisted selection and linkage map saturation.

In Brazil, several activities have been undertaken on *Musa* Genomics, including the complete sequencing of five 'Calcutta 4' BAC clones (that together account for approximately 525 Kb) and the partial sequencing of full-length enriched cDNA libraries from leaves of 'Calcutta 4'. Pairs of primers were designed to amplify 12 distinct SSR regions found in the sequences generated from these activities. Studies are underway to determine the level of polymorphism of these putative microsatellite markers in the segregating populations being generated by the banana breeding programme of Embrapa Cassava and Tropical Fruits, in Cruz das Almas, Bahia, Brazil.

P114 - Analysis of resistance gene analogs in 'Calcutta 4'

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Commercial banana varieties are cultivated in over 120 countries, generating an annual global production of around 100 million tonnes. Brazil represents the third largest producer, with a production area of 560 000 hectares and an annual production of 6 million tonnes. In 1998, the fungal pathogen *Mycosphaerella fijiensis*, the causal organism of black leaf streak disease, which causes premature fruit ripening, necrotic lesions leading to leaf area decomposition and yield losses of up to 50%[1] was reported for the first time in Brazil in the Amazon region[2]. Subsequently, the pathogen has spread to 6 states in the north of the country.

Specific recognition of plant pathogens, leading to programmed cell death, is controlled by resistance genes (R-genes). A number of R-gene subfamilies frequently possess conserved NBS (nucleotide binding site) and LRR (leucine rich repeat) domains[3]. The objectives of this study were to identify sources of resistance in 'Calcutta 4' through the analysis of resistance gene analogs (RGAs) using degenerate primers designed from highly conserved amino acid domains (NBS and LRR), and later via full length sequencing of positive BAC clones. To date, specific PCR amplification from genomic DNA targeting P-loop and GLPL amino acid motifs within the NBS domain has generated 15 sequence clusters that, based on BlastX analysis with a non-redundant NCBI protein database, show homology to resistance genes and RGAs from *Musa acuminata*, *Oryza sativa*, *Elaeis guineensis*, *Theobroma cacao*, and *Arabidopsis thaliana*. To date, PCR amplification, using a second set of degenerate primers designed from conserved amino acid motifs within non-Tir type NBS (P-loop, kinase 2, RNBS, GLPL) and LRR domains from monocot protein databases, has resulted in amplification of PCR products of expected size that are currently being sequenced. All RGAs will subsequently be characterized via reverse northern blots, in order to determine whether they represent gene regions, and candidate sequences will be labelled for use as probes against the 'Calcutta 4' BAC library.

1. Mourichon X., J. Carlier, E. Fouré. 1997. Sigatoka leaf spot diseases. *Musa* Disease Fact Sheet No. 8. INIBAP, France, 4pp.

2. Cordeiro Z. J. M., A. P. Matos, S. de O. de. Silva. 1998. La Sigatoka negra en Brasil. INFO*MUSA* 7:30-31.

3. Meyers B.C., A.W. Dickerman, R.W. Michelmore, S. Sivaramakrishnan, B.W. Sobral, N.D. Young. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant Journal 20:317-332.

P115 - Identification and cloning of disease resistance gene candidates from the local cultivar 'Jari buaya'

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Pests and diseases constitute one of the major constraints in the development of a sustainable banana industry in the country. Current control practices rely heavily on the use of chemicals and as such increase production costs. The use of cultivars resistant to the major diseases is the key to a more profitable banana industry. Enhancing resistance is therefore one of our main priorities but is not without challenges since many aspects of host-pathogen interactions are poorly understood. This study aimed to identify local banana cultivars that are resistant to *Fusarium oxysporum* f.sp. *cubense* race 4.

'Jari buaya' shows a high level of resistance to race 4 in the field, probably as a result of an incompatible interaction. Based on an analysis of the available resistance gene products, several DNA probes were generated by PCR. Resistance gene candidates and kinases were sequenced and compared with DNA sequences in the Genbank. Clones that revealed significant similarities to known resistance genes and analogues were further examined for conserved domains, unique motifs, expression behaviours and possible functions.

P116 - Small heat shock proteins in Calcutta 4 in response to temperature stress

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In 2001, INIBAP launched the Global *Musa* Genomics Consortium, which aims to develop freely accessible resources for *Musa* genomics and to use the new knowledge and tools to enable targeted conventional breeding and transgenic strategies. The development and characterization of full-length enriched cDNA libraries of *Musa acuminata* var. 'Calcutta 4' is one of the several activities undertaken in Brazil. Total RNA was extracted from banana leaves submitted to cold (5 to 25 °C) and hot temperatures (25 to 45°C). Two cDNA libraries were constructed, using the Creator™ Smart™ cDNA Library Construction Kit, and were characterized at the DNA Sequencing Platform at Embrapa Genetic Resources and Biotechnology. The characterization of these libraries has revealed six complete ORFs annotated as Small heat shock proteins (sHSPs). Small heat shock/alpha-crystallin proteins are defined by conserved sequences of approximately 90 amino acid residues. We report the functional characterization of *Musa acuminata* sHSPs produced under stress conditions.

Sequence and structural analyses show that the secondary structure of small heat shock/alpha-crystallin proteins is predominately beta-pleated sheet. The multiple sequence alignment revealed that the conservation of the amino acids A and Q (at residues 51 and 52) are noteworthy. The consensus regions I and II and the glutamine residue were conserved in all plant sHSPs, and it was suggested that this is the site of the transit peptide cleavage for all sHSPs. Features, such as the conserved arginine residue within the alpha-crystallin domain, were also observed in *Musa* sHSPs. Phosphorylation sites were predicted for small heat shock/alpha-crystallin proteins. It is known that sHSPs are expressed in plants under stress only, indicating that the ones found in *Musa* are a stress response to heat in its chaperone activity to stabilize the molten globule state of several constitutive cytosolic proteins. Further structural characterization and molecular modeling of these sHSPs from *Musa* is in progress.

P117 - Isolation of highly repetitive DNA sequences in *Musa acuminata* using reassociation kinetics

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Although the nuclear genome of *Musa acuminata* is relatively small (1C ~ 600 Mbp), experience with other sequenced genomes suggests that a large proportion (about 75 per cent) is composed of repetitive DNA sequences. Previous studies confirmed the presence of all major classes of such sequences, including retrotransposons. Compared to sequences coding for structural genes, repetitive DNA sequences are generally under less stringent control and evolve more rapidly. This makes them an ideal source of molecular markers and tools to study genome evolution. Repetitive DNA sequences may alter the expression of neighbouring genes and some of them play a major role in chromosome behaviour. Until now, only a limited set of repetitive sequences have been isolated from *Musa* but little is known about their genomic distribution and the long range organisation of chromosomes. The aim of this study was to isolate highly repetitive DNA sequences of *M. acuminate.*

Reassociation kinetics, also called C_0t analysis, is a powerful biochemical technique, which facilitates fractionation, cloning and characterization of various types of repetitive DNA sequences. During the course of this work, genomic DNA was isolated from *M. acuminata* 'Calcutta 4', sheared to ~ 300 bp fragments and subjected to C_0t analysis. A DNA library enriched for highly repeated DNA sequences was constructed from a low C_0t fraction (C_0t=0.05–0.1). The library, consisting of 7296 clones, was screened with probes for ribosomal DNA (*Radka*1 and *Radka*2) and other known repetitive *Musa* DNA sequences previously characterized.

In total, 192 anonymous DNA clones that gave no signals after hybridisation with the known sequences were chosen for sequencing. Dot-plot analysis was used for comparing individual sequences and for nucleotide analysis of each sequence. This analysis was also used to identify tandemly repeated DNA sequences such as satellite DNA, minisatellite and microsatellite sequences, which represented 20 per cent of sequenced clones. BLAST and FASTA homology searches revealed that, in addition to tandem repeats, the library was mostly composed of other highly repeated sequences such as retrotransposons. Forty-two percent of the sequences were novel and did not share a homology with known DNA sequences in other organisms. Southern-blot hybridization was used to determine the number of copies of highly repetitive DNA sequences per haploid genome of *M. acuminata*. Sequences displaying interesting properties were physically mapped by fluorescence *in situ* hybridisation on metaphase chromosomes of *M. acuminata*.

This work significantly expands the knowledge of the repetitive fraction of the *Musa* genome and long range organisation of the chromosomes. The repetitive DNA clones are being used to study genome evolution and diversification within the genus *Musa*.

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P118 - Standardizing a polymerase chain reaction test to detect banana streak virus

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Of the viral diseases that affect bananas, banana streak disease caused by the banana streak virus (BSV) is one of the most important. The diagnosis is often unreliable as the symptoms are variable and can be confused with those of banana mosaic. Serologically, BSV is moderately immunogenic and individual antisera often fail to detect some BSV isolates[1]. A polymerase chain reaction (PCR) test using the primers designed from a conserved domain of the viral genome would be useful in rapidly and reliably detecting BSV. In this study, an attempt was made to standardize PCR conditions, i.e. quantity of the DNA template, annealing temperature and the number of cycles required for amplification of BSV from infected samples of banana.

Specific primers were manually designed from the sequence data of the Onne isolate BSV (Genbank accession No. AJ 002234)) from the region corresponding to the conserved domain of reverse transcriptase and RNase H. PCR amplification was performed in 50 µl reaction mixtures using 1 µM of primer, 200 µM each of dNTPs, 0.05 unit/µl of Taq DNA polymerase, 1 x reaction buffer, 1.5 mM of $MgCl_2$ and varying quantities of DNA template (ranging from 0.5µl to 10µl) from symptomatic and non-symptomatic banana plants. Using the gradient function of thermocycler (Biometra model T - Gradient Thermoblock, Germany), a temperature gradient of annealing temperature of 53^{o}C to 63^{o}C (53, 53.9, 56.2, 57.4, 58.6, 59.8, 60.9, 62 and 63^{o}C) was set. Different cycles of amplification (20, 25, 30 and 35 cycles) at 94^{o}C were also tried. The other parameters of PCR amplification were: one cycle of 94^{o}C denaturation for 4 min, varying cycles (20, 25, 30 and 35) each at 94^{o}C for 30 s, gradient temp (53^{o}C to 63^{o}C) for 30 s and 72^{o}C for 30 s, followed by one cycle of final extension for 10 min.

The results showed that there was amplification even at a concentration of 1µl, but 5µl of DNA template showed a very clear band. In the case of annealing temperature, the intensity of amplification was maximum at 58.6^{o}C compared to the other temperatures. Thirty cycles gave optimum amplification. It is concluded that the PCR conditions standardized in this study, using the primers corresponding to the conserved domain of reverse transcriptase and RNase H gene of BSV, can be used to more reliably detect BSV in infected samples.

1. Lockhart B.E. L. & N.E. Olszewski. 1993. Serological and genomic heterogeneity of banana streak badnavirus: Implications for virus detection in *Musa* germplasm. Pp. 105-113 *in* Breeding banana and plantain for resistance to disease and pests (J. Ganry, ed.). INIBAP, Montpellier, France; CIRAD, Montpellier, France.

P119 - Cloning the coat protein gene of the cucumovirus causing mosaic disease in India

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Banana is one of the most important fruit crops of India. It is mainly grown by smallholders and as such plays a major role in food security and income generation. In spite of its importance, the yields remain very low and diseases, especially viral ones, pose a major threat not only to production but also to germplasm movement and to the exchange of planting material. Banana mosaic disease, also known as infectious chlorosis, is now emerging as a serious disease affecting crop yield[1] and is widespread in Kerala, the southernmost state of India. This disease is caused by the cucumber mosaic virus (CMV). In Kerala, intercropping banana with cucurbitaceous vegetables is a very common practice, which has lead to an increase in the disease.

The first symptoms, lemon yellow streaks or patches, appear on young folded leaves or newly expanded leaves. These chlorotic patches later turn brown in colour. The leaves show inward rolling and malformation in some cases. The electron microscopy of infected leaf samples revealed the presence of spherical shaped particles of about 29 nm in diameter that are morphologically similar to the cucumber mosaic virus.

A RT-PCR protocol for detecting CMV from infected samples was standardized using the upstream primer (5'CATCGACCATGGACAAATCTGAATCAAC) and the downstream primer specific to the coat protein gene (3'CTCTCCATGCGTTTAGTGACTTCAGCAG). Amplification was performed in an automated thermal cycler (Power Block II, Ericomp. Inc., San Diego, CA, USA). An amplicon of about 750 bps corresponding to the coat protein gene was obtained. It was cloned in a pGEM-T vector (Promega) by A-T cloning and sequenced.

The sequencing revealed an ORF of 657 nucleotides coding for 218 amino acids, starting with methionine and ending with threonine. The sequence was analysed using the Clustal W programme and a phylogenetic analysis revealed that it was closely related to an isolate from Israel. The clones could be used as molecular probes for the detection of CMV in bananas and as a diagnostic tool for the screening of suckers distributed to farmers. They could be further exploited to induce resistance in genetically transformed bananas.

1. Estelitta S., T.C. Radhakrishnan and T. Sheela Paul. 1996. Infectious chlorosis disease of banana in Kerala. Info*Musa* 5(2):25-26.

P120 - Diversity in genomic distribution of ribosomal DNA and nuclear genome size in *Musa*

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The taxonomy of *Musa*, which comprises about 50 species, has never been fully resolved and remains a subject of debate. Traditional approaches based on the analysis of morphology and chromosome number have recently been supplemented by analyses of the DNA, such as ribosomal gene spacer length, RFLP and AFLP. These studies revealed shortcomings of the current *Musa* classification. Basic features of the nuclear genome and karyotype are known for only some species. For example, genome size has been estimated for *Musa acuminata* and *Musa balbisiana* and only a few other species. Ribosomal RNA (rRNA) genes (45S rDNA and 5S rDNA) are organized in tandemly repeated units localized at a few discrete chromosomal sites, an arrangement that facilitates their visualization. We have previously shown that fluorescence *in situ* hybridization (FISH) with probes for ribosomal genes can be used to identify a subset of *Musa* chromosomes. However, until now only *M. acuminata* and *M. balbisiana* and their hybrids have been studied with this technique. The present study was undertaken to determine the nuclear genome size and the genomic distribution of 5S and 45S rDNA in nineteen diploid accessions representing four traditionally accepted sections of *Musa* (*Eumusa*, *Rhodochlamys*, *Callimusa* and *Australimusa*), in order to clarify the relationship between the species and sections. *Ensete gilletii* was included as an outgroup.

In *Eumusa* (x=11), 2C DNA content ranged from 1.130 to 1.377 pg, with *M. balbisiana* having the lowest DNA content of all sections. *Musa beccarii* (x=9), a representative of *Callimusa*, had the highest 2C nuclear DNA content (1.561 pg). Species belonging to *Rhodochlamys* (x=11) and *Australimusa* (x=10) had 2C DNA contents ranging from 1.191 to 1.299 pg and from 1.435 to 1.547 pg, respectively. *E. gilletii* (x=9) had 2C DNA content of 1.210 pg. The number of 5S rDNA loci in *Musa* varied from 4 to 8 per diploid cell.

While different numbers of 5S loci were observed within *Eumusa* and *Rhodochlamys*, four 5S loci were observed in all the accessions of *Australimusa*. The number of 45S rDNA loci was conserved within individual sections. A hierarchical cluster analysis of genome size, number of chromosomes and 45S rDNA sites suggests a close relationship between *Rhodochlamys* and *Eumusa*. *Australimusa* was clearly separate, as were *M. beccarii* and *E. gilletii*. Within the *Eumusa*-*Rhodochlamys* group, *M. balbisiana*, *Musa shizocarpa* and *Musa ornata* formed distinct subgoups, clearly separated from the accessions of *M. acuminata*, *Musa mannii*, *Musa laterita* and *Musa velutina*, which formed a tight subgroup. These results expand the knowledge on genome size and genomic distribution of ribosomal DNA in *Musa* and *Ensete*. They aid in clarifying the taxonomical classification of *Musa* and show a need to supplement the analyses on DNA sequence level with cytogenetic studies.

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P121 - Integration of banana streak virus genome in *Musa* germplasm with B genome

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Banana streak badnavirus is a pararetrovirus that infects bananas and plantains in India. This virus integrates in the genome of the plant and stress may trigger the episomal expression of the virus and cause infection. The virus is a problem for breeders because it is transmitted to the progeny when one of the parents is infected. Moreover, diploids need to be checked for viral integrants before they can be used in polyploidization. An attempt was made to detect integrants and BSV infection by PCR.

Musa leaf tissues were collected from the NRCB genebank and the DNA extracted[1.] for use in the PCR[2]. Amplification was performed in Eppendorf Master cycler gradient PCR system. The first cycle of denaturation lasted 5 minutes at 94°C and was followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 60 seconds at 72°C, and completed with a cycle of 10 minutes at 72°C. In this investigation B genome accessions from the Mysore and BB groups, commercial cultivars, exotic cultivars such as Saba, FHIA-3, 'Pisang Ceylan' and a diploid cultivar were tested for the presence of BSV integrants.

The result showed that out of the seven BB accessions tested, three had integrants (1.5 kbp) and four were positive for RSR primers. Among commercial varieties tested only 'Poovan' (AAB) had integrants. Out of the seven Mysore accessions tested, accessions 586, 653 and one from a collection in Manadu, Tamil Nadu were positive for integrants. 'FHIA-3' and 'Saba' did not have any integrant, but one out of four samples of 'Pisang Ceylan' tested positive. Out of five hybrids tested for BSV, four were positive for virus specific primers but not for integrants. Implications of BSV infection in Indian germplasm are discussed in detail.

1. Selvarajan R., V. Balasubramaniam, S. Dhayakar, S. Uma, Y.S. Ahlawat and S. Sathiamoorthy. 2002. Molecular diagnosis of Banana bunchy top nano virus (Indian isolate) coat protein gene by Polymerase Chain Reaction. P. 142 *in* Global conference on banana and plantain, 28-31 Oct. 2002, Bangalore, India.

2. Dallot S, P. Acuna, C. Rivera, P. Ramirez, F. Côte, B.E.L Lockhart and M.L. Caruana. 2001. Evidence that the proliferation stage of micropropagation procedure is determinant in the expression of Banana streak virus integrated into the genome of the FHIA-21 hybrid (*Musa* AAAB). Arch Virol. 146:2179-2190.

P122 - Isolation and characterization of post-transcriptional gene silencing associated genes

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In plants, post-transcriptional gene silencing (PTGS) forms the basis of virus-induced gene silencing (VIGS), suggesting an important role in pathogen resistance. This involves a sequence specific degradation of RNAs, a phenomenon triggered by double-stranded RNAs (dsRNAs) and called RNA interference in animals, PTGS in plants and quelling in filamentous fungi. Many plant viruses encode for suppressors of PTGS that are essential in pathogenesis. These determinants of virulence can be masked by host mutation in silencing pathways. PTGS has also been linked to the control of endogenous transposable elements. In this study, we attempted to isolate and characterize CARPEL FACTORY, an ortholog of DICER in animals, which cleaves dsRNA into small interfering RNA, or microRNA, and ARGONAUTE, one of the components that form the RNA-induced silencing complex. The role these genes play in banana gene silencing and pathogen resistance will be discussed.

P123 - A binary bacterial artificial chromosome genomic library of the *Musa acuminata* AA cv. 'Tuu gia' resistant to black leaf streak disease

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We have constructed a binary bacterial artificial chromosome (BIBAC) genomic library of the diploid *Musa acuminata* (AA) cv. ´Tuu gia', which is resitant to black leaf streak disease (BLSD). In an artificial inoculation experiment using 63 strains of *Mycosphaerella fijiensis* collected in various countries, it was reported[1] that of the 20 wild and cultivated bananas tested, only 'Tuu gia' was resistant to all 63 strains. The resistance of 'Tuu gia' apparently does not break down, even under the pressure of highly virulent strains.

The library was established from one ligation with a 4:1 vector:insert ratio and consists of 30 920 clones. The insert size of 120 randomly selected clones indicated a mean insert size of 100 kb. The frequency of inserts with internal *Not*I sites was 57%. All the detected clones had inserts. The majority of insert sizes fell into the range of 100 to 119 kb, with more than 50% of inserts larger than 100 kb. Considering that the size of the diploid AA banana haploid genome is estimated to be 600 Mb[3], this BIBAC library represents five times the haploid genome. We have estimated that highly repetitive DNA is found in 27% of the clones out of a total of 4608 (3 x 4 x 384) random clones (3 filters).

We found that 31 clones out of the 3072 (2 x 4 x 384) analysed clones were positive for chloroplast cp DNA. Thus the presence of cpDNA was estimated to be 1%. The mtDNA content was found to be 0.9%. This content organelle DNA is judged to be acceptable and is an indication of the quality of the library.

The cultivar 'Tuu gia' is highly sterile and therefore genomic applications that rely on genetic mapping, such as map-based cloning, cannot be considered. However, using other approaches for gene or gene cluster discovery, such as the use of resistance gene analogues as probes, we consider that this transformation-ready large insert genomic library will be a useful resource for the global *Musa* research community.

1. Fullerton R.A. and T.L. Olsen. 1995. Pathogenic variability in *Mycosphaerella fijiensis* Morelet, cause of black Sigatoka in banana and plantain. New Zealand Journal of Crop and Horticulture Science 23:39-48.

2. Ortiz R. 2000. Understanding the *Musa* genome: an update. Acta Horticulturae 54:157-168.

3. Dolezel J., M. Dolezelova, F.J. Novak. 1994. Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*) Biol. Plant. 36:351-357.

P124 - Construction and characterization of a bacterial artificial chromosome genomic library of *Mycosphaerella fijiensis*

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For the first time, a Bacterial Artificial Chromosome (BAC) genomic library was constructed from an isolate of the most important fungal pathogen of banana, *Mycosphaerella fijiensis*. Using a novel technique, which does not require sphareoplasting, high molecular weight DNA (megabase) was isolated from mycelia of *M. fijiensis*, partially digested with the restriction enzyme *Hind* 111, subjected to double size selection, electroeluted and ligated in the vector pCC1BAC (Epicentre). The library consists of 1920 clones. We analysed 100 clones selected at random for mean insert size, frequency of clones without inserts and percentage of clones with internal *Not*1 sites.

Our results indicate that mean insert size of the inserts is 90 kb and that 84% of inserts are between 80 Kb and 100 Kb, 4.8% of the clones do not have inserts and 32% of the inserts have 2 or 3 internal *Not* 1 sites. This represents 6 times the genome, if the genome minimum size is considered to be 28 Mb (estimated by electrophorectic karyotype for this isolate). This gives a 99% theoretical probability that all sequences will be represented. However if the maximum likely genome size is considered to be 40 Mb (expected size for this fungus) then this represents 4 times the genome. The clones were picked into five 384 well microtiter plates, replicated twice and stored at -80°C. As a priority for the *Mycosphaerella* Genomics Consortium, this library will be initially used to isolate the MAT idiomorphs (mating type genes) and will also be useful for physical mapping by restriction digest fingerprinting and construction of contigs.

P125 - Relationship between the aggressiveness of *Mycosphaerella fijiensis* isolates and the susceptibility of the cultivar from which it was isolated

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Five monoascosporic isolates of *Mycosphaerella fijiensis* (the causal agent of black leaf streak disease*)* were isolated from lesions on the susceptible *Musa acuminata* Cv 'Grande naine' (AAA) and six monoascosporic isolates were isolated from lesions on the tolerant cultivar TMX5295-1. All isolates were inoculated to 'Grande naine' under greenhouse conditions.

Disease development time was longer when using isolates obtained from the susceptible cultivar compared to those from the tolerant cultivar, indicating that isolates obtained from the tolerant cultivar were more aggressive. When all the isolates were grown in liquid medium, there was a highly significant difference in the pack cell volume (PCV), fresh weight and dry weight of the isolates from the susceptible cultivar compared to the isolates from the tolerant cultivar. All the isolates from the susceptible cultivar had significantly higher PCV, dry weight and fresh weight than those obtained from the tolerant cultivar. These results suggest that there is a physiological cost associated with being the more aggressive phenotype. Pulsed field gel electrophoresis karyotyping of 3 isolates differing in aggressiveness revealed karyotype polymorphisms. However there was insufficient evidence to indicate whether this karyotype polymorphism was correlated with the aggressiveness of the isolates. These results suggest that the deployment of tolerant cultivars might have an impact on the evolution of the pathogen.

P126 - *Musa* collecting in Maluku and Papua

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Indonesia is a center of origin and diversity of the genus of *Musa*. Great diversity of *Musa* (wild or cultivated) can be found from Aceh (Sumatera) to Papua (Irian Jaya). Indonesian Fruit Research Institute has performed two collecting missions in collaboration with INIBAP. The first mission was carried out in Maluku Islands from 18 November to 14 December 1996.. Maluku Islands lies between 124 to 134.8°E and 2.65°N to 8.35°S and is composed of three large islands and some small islands. Four wild accessions and 24 cultivars were collected.

The second mission was conducted in Papua between 11 February and 4 March 2002. Papua lies between 131 to 141°E and 0°to 9°S and the highes peaks attain 3100 m. Banana cultivation is found throughout the island until 2500 m. Some ethnic groups use banana as a staple food. Papua is adjacent to Papua New Guinea, which has many different diploid clones. Many of these clones are also found in Papua. In the second collecting mission, 75 accessions were collected. Forty-nine of these accessions have so far been characterized.

There is significant variation between the accessions. ‘Kaikeja’ is pink, while ‘Neij mom’, ‘Mne mowa’ and ‘Kilita’ are light yellow and ‘Teget molo’ is purple. Unique bunch characteristics were observed on ‘Ambonaee’ (AAA), which had two bunches in Papua, and ‘Sepatu amora’ (ABB), which had no male bud in Seram Island (Maluku). ‘Honggambu’, ‘Iren’, ‘Keja’, ‘Urelu’, ‘Wanggonak’ and ‘Wundi’ were whitish green. ‘Pisang tongkat langit’, a Fe’i banana from Maluku and Papua, ‘Ndinyale’ and ‘Awomen’ had orange pulp, indicating high levels of β-carotene. Some ABB bananas such ‘Pisang boi’, Sepatu, ‘Neij amusta’ (Bluggoe), ‘Kumro’ and ‘Selayar’ (Pisang awak) are cooking bananas. ‘Pisang jarum’ is a commercial dessert banana in Maluku, whereas ‘Pisang raja’ is a commercial cooking banana in Seram Island. Some native cultivars of Papua are similar to bananas in Papua New Guinea. *Musa lolodensis*, *Musa shizocarpa* and *Musa acuminata* were found in the district of Manokwari in West Papua province.

The collected germplasms are maintained at the Indonesian Research Institute for Fruits as *in vitro* plants, in greenhouses and in the field. They are currently being characterized morphologically, agronomically and with regards to their various uses. Some of the data have already been entered in MGIS.

P127 - Improving ‘Pisang raja’ (AAB) through selection

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Malaysia is the home of many varieties of bananas. This diversity opens up opportunities for breeders and farmers to select for higher yield, disease tolerance and fruit quality. ‘Pisang raja’ (meaning king banana) is a dual-purpose cultivar. It can be eaten as a dessert banana, as is popularly done by the Chinese community, or cooked, which is very popular among Malays. Its yield, however, is low compared to other cooking varieties such as ‘Pisang nangka’, ‘Pisang abu’ and ‘Pisang awak’. Time to flowering is also long, 9-10 months, compared to the dessert bananas ‘Pisang mas’ (6 months), ‘Pisang berangan’ (7 months) and Cavendish (6-7 months). Being tall is another negative feature and like all commercial bananas, ‘Pisang raja’ is also susceptible to Fusarium wilt.

‘Pisang raja’ accessions were collected throughout peninsular Malaysia in some 90 locations. From 1 to 3 mats were collected per location with 1-4 suckers per mat. Planting was carried out under irrigation at MARDI, Serdang, using standard cultural practices. Time to flowering, plant height and girth, bunch weight, fruit length and tolerance to Fusarium wilt were recorded. The average yield was 14-16 kg, time to shooting was 250-300 days and plant height was 250-300 cm.

Above average accessions were selected from this first round of evaluation. One accession, 51-B, from Raub, Pahang, had a bunch weight of 16 kg for the mother-plant crop, that increased to 17 kg and 23 kg in the first and second ratoon crops, respectively. This accession was still free of Fusarium wilt after three production cycles. Plant height, however, had increased from 280 cm to 399 cm by the second ratoon crop. Time to first flowering was 232 days. Other potential accessions include ‘Pisang raja sanggang’ and ‘Pisang raja ulu dong’. The selected accessions were multiplied *in vitro* and further selection was made for higher yields, early fruiting and tolerance to Fusarium wilt.

P128 - Agronomic potential of new 'Dwarf Cavendish' clones

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The majority of banana selection and breeding programmes are trying to produce cultivars that are resistant or tolerant to fungi, mainly *Mycosphaerella fijensis* (the causal agent of black leaf streak disease) and *Fusarium oxysporum* f. sp. *cubense* (the causal agent of Fusarium wilt). In the case of the Canary Islands, where black leaf streak disease is absent and the incidence of Fusarium wilt is low, breeding focuses on producing highly productive dwarf clones that would allow the local banana growers to compete with regions where the production cost is lower. Although the Canary Islands are probably the area with the highest productivity in the world, selection work has been aimed at producing better quality clones in greater quantities. These new clones would also be potentially interesting for other regions, especially subtropical areas. We will describe the methodology used to select the clones and their characteristics. Some of the clones are already planted on a large scale by farmers. The results illustrate the potential of 'Dwarf Cavendish', a generally neglected cultivar in breeding programmes.

Session 2

Plant protection

Oral presentations

Keynote lecture: Population genetic structure and dispersal of the fungal pathogen of bananas *Mycosphaerella fijiensis*

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The worldwide destructive epidemic of the fungus *Mycosphaerella fijiensis* on bananas started recently, spreading from Southeast Asia. *M. fijiensis* is an haploid and heterothallic ascomycete fungus and it spreads through three modes[1]. The wind-borne spread of ascospores produced during the sexual reproduction is believed to be limited to few hundred kilometers[2]. Conidia produced during asexual reproduction might be more involved in short-distance dispersal on the plant and to nearby plants. Populations and epidemiological studies of *M. fijiensis* have been undertaken (i) to provide information on the level and distribution of variability, (ii) to infer on dispersal process of *M. fijiensis* and (iii) to evaluate the relative importance of evolutionary factors on the pathogen.

Population structure of *M. fijiensis* was analysed from global to plant scales using molecular markers[3,4]. The results indicate that a high level of genetic diversity is maintained at the plantation and plant levels. The loci were at gametic equilibrium in most of the samples analysed, supporting the hypothesis of the existence of random-mating populations of *M. fijiensis*, even at the plant level. Southeast Asia has the highest level of genetic diversity, supporting the idea that the pathogen originated in this region.

Founder effects were detected at the global and continental scales. Genetic differentiation values between populations decreased with the geographical scale considered, from a high level at global scale (F_{st} = 0.52 between continents) to a non-significant level at the local scale (F_{st} = 0 between nearby plantations) (Figure 1). An isolation by distance analysis was conducted in Costa Rica and Cameroon within a production area (around 300 km long) to estimate gene flow and study the dispersal process of the pathogen (D. Coste and colleagues, unpublished results). A strong isolation by distance was detected in both countries, suggesting important dispersal of ascospores on short distances. These results are consistent with those obtained from a direct analysis of disease gradient with a mean dispersal distance of ascospores estimated at a few dozen meters (C. Abadie and colleagues unpublished results).

The results obtained could reflect the relative importance of dispersal through infected plant materials and ascospores in relation to geographical scales (Figure 1). The relative importance, on the epidemiology of *M. fijiensis,* of ascospores and conidia dispersal over short distances on cannot be evaluated from the available data. At a global scale, the introduction of the disease in various continents is probably the result of rare movements of infected plant material. The spread of the disease within a continent may result either from limited ascospores dispersal over a few hundred kilometers or from the movement of infected plant material. The dispersal of *M. fijiensis* over long distances appears stochastic, resulting in founder effects, a limited gene flow between established populations and consequently in a high level of genetic differentiation between them. The spread of ascopores may progressively increase when geographical scale decreases from continental to local scales. Thus, the dispersal of *M. fijiensis* may become more and more gradual leading to a diminution of genetic differentiation and isolation by distance at the intermediate scale (a few hundred kilometers).

Population structure of *M. fijiensis* is now better known. However, such studies should be conducted in Southeast Asia for the different *Mycosphaerella* leaf spot pathogens in order to identify zones of co-evolution. The results to date also show the effects, on the population structure of pathogens, of genetic recombination, genetic drift and gene flow. A study is being developed at the local scale to evaluate the effect of the selection pressure exerted by the host on the pathogen[5].

1. Gauhl F., C. Pasberg-Gauhl and D.R. Jones. 2000. Black leaf streak. Disease cycle and epidemiology. Pp. 56-62 *in* Disease of bananas, Abaca and Enset (D.R. Jones ed.). CABI, Wallingford.

2. Parnell M., P.J.A. Burt and K. Wilson. 1998. The influence of exposure to ultraviolet radiation in simulated sunlight on ascospores causing black Sigatoka disease of banana and plantain. International Journal of Biometeoroly 42:22-27.

3. Carlier J., M.H. Lebrun, M.F. Zapater, C. Dubois and X. Mourichon. 1996. Genetic structure of the global population of banana black leaf streak fungus, *Mycosphaerella fijiensis*. Molecular Ecology 5:499-510.

4. Rivas G.G., M.F. Zapater, C. Abadie and J. Carlier. 2004. Founder effect and stochastic dispersal at the continental scale of the fungal pathogen of bananas *Mycosphaerella fijiensis*. Molecular Ecology 13:471-482.

5. Abadie C., A. El Hadrami, E. Fouré and J. Carlier. 2003. Efficiency and durability of partial resistance components of bananas against black leaf streak disease. Pp. 161-168 *in* Mycosphaerella leaf spot diseases of bananas: Present status and outlook. (L. Jacome, P. Lepoivre, R. Marin, R. Ortiz, R.A. Romero and J.V. Escalant eds). INIBAP, Montpellier, France.

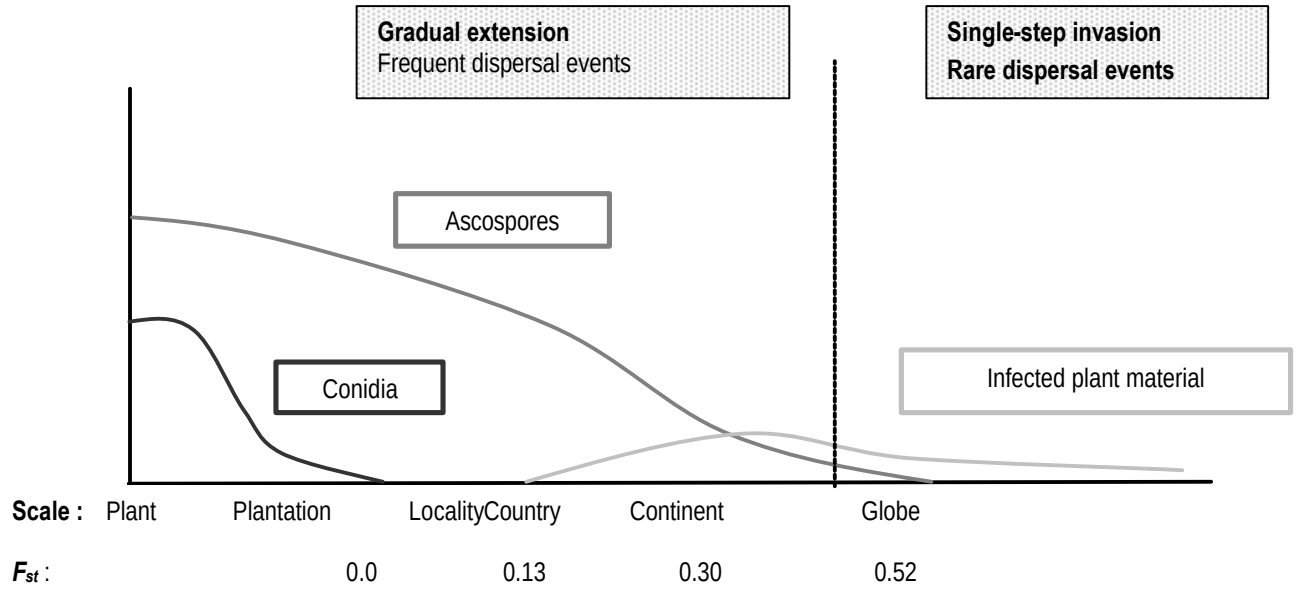

Figure 1. *Hypothetical relative importance of the three dispersal modes of the fungus* Mycosphaerella fijiensis *as a function of geographical scale and genetic differentiation between populations (estimated by* F_{st}*).*

Keynote lecture: Diseases and pests: A review of their importance and management

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Diseases and pests are among the most significant constraints encountered in *Musa* production. Regional differences occur in their prevalence and relative impact, and those caused by fungi are most noteworthy. Leaf spots caused by species of *Mycosphaerella* result in moderate to severe damage wherever significant rainfall occurs, and that caused by *M. fijiensis*, known as black Sigatoka or black leaf streak, is the most important. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense*, is a lethal and widespread problem and, depending on the race that is present, can impact diverse cultivars. Bacterial wilts caused by *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *musacearum* are also lethal, but less widely distributed. Bunchy top, caused by *Banana bunchy top virus*, is the most damaging virus-induced disease, but also has a limited geographic distribution. Nematodes are the most important pests, and *Radopholus similis* is the most widespread of those that cause serious damage. Finally, the weevil borer, *Cosmopolites sordidus*, is the most prevalent and important insect.

Options for the sustainable management of these problems are usually limited. Cultural measures are successful in certain situations (e.g. against the bacterial diseases), but are marginally so in others (leaf spots in areas with high rainfall). Chemical control is possible for only the leaf spots and pests. Although it can be effective, (especially for the leaf spots), its environmental impact and high cost restrict its use. In other cases, genetic resistance and cultural management are indicated. Resistance to the fungal diseases and *R. similis* has been identified and used in conventional breeding programs, but to a great extent does not exist for other pests and the bacterial and viral problems. In the latter situations, non-conventional approaches, in particular GM, have received considerable attention. The extent to which GM approaches are successful and whether consumers will ultimately accept GM fruit are presently unknown. Future threats and research priorities will be discussed.

Impact of *M. fijiensis* metabolites on banana antioxidant systems

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The injection of *Mycosphaerella fijiensis* culture filtrate extracts in banana leaf tissues induced the development of necrosis and modified the chlorophyll fluorescence profile. This biological activity was shown to be light dependent and was correlated with the performance in field tests of two reference genotypes ('Fougamou' as partially resistant and 'Grande naine' as susceptible). The plant's photosynthetic apparatus is suspected to constitute a target site for *M. fijiensis* toxins based on electron microscopy observations, such as the swelling of the susceptible 'Grande naine' chloroplasts in leaves injected with crude extracts of the pathogen), the light-dependence of the toxicity and the early effect on chlorophyll fluorescence profiles.

The purification and chemical characterization of *M. fijiensis* culture filtrate extracts revealed the presence of juglone, a molecule belonging to the main group of naphthoquinones, within the most toxic fraction. Injection of this purified metabolite in banana leaf tissues gave rise to similar necrotic and chlorophyll fluorescence profiles as the ones observed after the injection of total crude extracts. The electron exchange ability of isolated chloroplasts, as measured with Hill's reaction[1], was significantly inhibited by juglone or semi-purified fractions of the crude extracts, confirming the hypothesis of the photosynthetic apparatus as a target site for *M. fijiensis* metabolites.

Since oxidative events were thought to be responsible for the physiological damages caused by *M. fijiensis* metabolites in banana tissues, the interactions between juglone and some plant antioxidant systems were evaluated. Direct contact between juglone and ascorbic acid, the most abundant antioxidant in plants[2], resulted in the oxidation of the latter. This observation can be considered as a first proof of oxidative potential of juglone by decreasing global antioxidant capacity. The occurrence of oxidative mechanisms was also investigated at the level of the superoxide dismutase (SOD) patterns exhibited by the two reference cultivars after treatment with juglone. A repressive effect induced by juglone on one SOD isoform was detected in the susceptible cultivar 'Grande naine' whereas there was a stimulation of another SOD isoform by the same fungal metabolite in the partially resistant cultivar Fougamou. These preliminary results can be interpreted as demonstrating that juglone, a *M. fijiensis* metabolite, interacts with antioxidant systems to partly deprive bananas of their antioxidant capacity and as such lead to oxidative damages.

1. Allen J.F. and N.G. Holmes 1986. Electron transport and Redox Titration. Pp. 103-141 *in* Photosynthesis energy transduction. A practical approach (M.F. Hipkins and N.R. Baker, eds). IRL PRESS, Washington.

2. Smirnoff N. 2000. Ascorbic acid: metabolism and functions of a multi-facetted molecule. Current Opinion in Plant Biology 3:229-235.

Effect of Fusarium on plants precolonized with biocontrol agents

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Fusarium wilt of banana is a serious disease in southern India. Two important cultivars, 'Rasthali' and 'Ney poovan', are highly susceptible. Chemical control of this disease has not been successful and attempts to develop resistant clones with conventional breeding techniques have had limited success. Several studies have suggested that treatment with selected endophytic bacteria and fungi could sensitize plants to defend themselves against pathogen attack[1]. Previous work in our laboratory showed that inoculation of 'Ney poovan' with vesicular arbuscular mycorrhizal (VAM) fungi at planting increased plant height and nutrient uptake, and decreased time to fruiting and yield[2].

In the present study, we tested banana precolonized singly or in combination with VAM, *Trichoderma harzianum* and *Pseudomonas fluorescens* against Fusarium wilt under field conditions. An ELISA assay was employed to assess pathogen colonization and the efficacy of the inoculants. Polyclonal antibodies were raised in rabbits against *Fusarium oxysporum* f.sp*. cubense* (Foc) mycellium and spores (9.2×10^4 CFU/ml). Antiserum was purified by affinity column chromatography (Sigma) and the concentration of antibodies (0.353 mg/ml) determined.

Plants were treated in the field with 500 g of VAM (soil-based inoculum containing 40 spores/g of soil), *T. harzianum* (50 g) and *P. fluorescens* (50 g) and after 90 days were challenged with 50 g of Foc multiplied on sorghum seeds. Plants were scored for disease incidence and concentrations in roots were determined at monthly intervals with the ELISA assay. Root or corm tissue (0.5 g) in each treatment was tested using 1:500 dilutions of antibody in five replications. Foc populations were estimated with a standard regression of Foc mycelium and spore concentration vs unit protein against a BSA standard. Foc concentrations were reduced in roots that had been precolonized with biocontrol agents. Compared with the inoculated check, the observed reductions after 7 months were 70% (VAM + *T. harzianum),* 69% *(T. harzianum*), 68% (VAM), 65% (*P. fluorescens*) and 54% (VAM + *T. harzianum* + *P. fluorescens*).

1. Kloepper J.W. 1993. Plant growth-promoting rhizobacteria as biological control agents. Pp. 255-274 *in* Soil Microbial Technologies (B. Metting, ed.) M. Dekker Inc., New York.

2. Sukhada M. 1995. Utilization of VAM fungi in banana cultivation. Proceeding, National Seminar on Mycorrhiza, Biofertilizers of the future held at New Delhi, on 29-31 March 1995.

Occurrence of nematodes on common cultivars in South Africa

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The predominant nematode species found on bananas in South Africa are *Radopholus similis, Pratylenchus coffeae, Helicotylenchus multicinctus, Meloidogyne incognita* and *Meloidogyne javanica.* A survey was conducted in the three main banana-producing areas of South Africa namely Onderberg, Hazyview and South Coast of Kwazulu/Natal. Nematode samples were taken from various cultivars such as 'Chinese Cavendish' (AAA), 'Dwarf Cavendish' (AAA), 'Grande naine' (AAA), 'Williams' (AAA) and 'Goldfinger' (AAAB). *R. similis, P. coffeae*, *Helicotylenchus* spp. and *Meloidogyne* spp. occurred in the three areas with the latter two being most abundant. Although *R. similis* is the most damaging nematode species on bananas and was found in all three banana producing areas, mean numbers were low. Other species were *Rotylenchulus reniformis, Paratylenchus minutus*.and *Paratrichodorus minor.*

'Chinese Cavendish', 'Williams', 'Grande naine' and 'High noon' (AAAB) were also tested in the greenhouse for resistance to *R. similis* and *Meloidogyne* spp. 'Grande naine' was more tolerant to *R. similis* and 'Chinese Cavendish' seemed to be most susceptible to *R. similis*. It was found that an increase of *Meloidogyne* numbers did not have a negative effect on the growth of banana plants in the greenhouse. 'Chinese Cavendish' and 'High noon' had high gall ratings and nematode numbers in the roots, but their root systems appeared healthy.

Incidence of cucumber mosaic virus in Nigeria

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A survey to determine the incidence of natural cucumber mosaic virus (CMV) infection in *Musa* spp. in farmer's fields and household gardens was conducted in eleven of the major plantain and banana growing states of southeast and southwest Nigeria between 1998 and 2000. Leaf samples exhibiting virus-like symptoms were collected from 917 *Musa* spp. plants. The samples were tested for CMV by using polyclonal antibodies in a dot blot immunoassay and 619 tested positive for CMV.

The most common symptoms associated with CMV infection were interveinal chlorosis and chlorotic streaks. These were seen in 30% and 27.5% of the samples respectively. Other symptoms seen in less than 10% of the plants included unusually thick veins on the lamina, puckering and crinkling, chlorotic flecking, chlorosis, necrotic spots and mosaic. A combination of symptoms was seen in about 3.4% of samples. The morphology of the causal agent was confirmed by immunosorbent electron microscopy as a 29 nm diameter isometric virus. The identity of the virus was confirmed by the reverse-transcriptase polymerase chain reaction using a pair of CMV-specific primers. The expected product size of 500 bp was obtained. This study confirms that CMV causes a large proportion of the virus-like symptoms seen on *Musa* spp. in southeastern and western Nigeria. The implications of this with respect to weeds and alternative crop hosts are discussed.

Genetic diversity of AAA cultivars and banana bunchy top virus in Vietnam

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A collection of 124 cultivars belonging to the AA, BB, AAA, AAB, ABB, AAAA and AABB groups was conducted in Vietnam. Molecular marker techniques (RAPD, AFLP, isozymes) were used to screen the genetic diversity of 18 AAA cultivars. We used 50 primers 10-mer random of Operon, 15 combination of primer pairs AFLP and two isozyme systems, eseterase and peroxydase. The results showed a high level of diversity among AAA cultivars. The highest similarity was 78% and the lowest 35%. The phylogenetic tree constructed by using the NTsyspc 2.1 programme showed that cultivars collected from the same area tended to be grouped in the same branch of the tree.

Banana plants infected with the banana bunchy top virus were used to extract viral DNA. A section purported to contain putative genes encoding for the virus protein coat was sequenced. The sequence showed 95% similarity to the one in previous reports.

Phylogenetic diversity of *Mycosphaerella* leaf spot diseases

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Mycosphaerella leaf spot diseases are the biggest constraint to commercial banana production throughout the world. The three main causal agents are *Mycosphaerella musicola* (Sigatoka disease, also known as yellow Sigatoka), *Mycosphaerella fijiensis* (black leaf streak disease, BLSD, also known as black Sigatoka) and *Mycosphaerella eumusae* (eumusae leaf spot disease). These species are closely related and have very similar morphology. It is generally easier to distinguish them by examining them microscopically. A molecular diagnostic designed in the Internally Transcribed Spacer (ITS) regions aids in the early and accurate distinction of BLSD from Sigatoka disease. This test was crucial in the eradication of BLSD from Australia. To validate this test, phylogenetic analysis of the ITS region was performed on 225 samples of *Mycosphaerella* spp. from around the world. A high level of diversity between these species was revealed, each species forming distinct clades composed of a number of sub-clades (Figure 1). This analysis also helped trace the origins of BLSD in Australia.

Seven distinct clades were identified from 111 samples of Sigatoka disease, indicating several geographically distinct strains. The nucleotide variation between the clades Yellow 1 to Yellow 6 ranged between 4 and 14 nt. Clade 7 was isolated from the clades of the three species and it may be a previously undescribed species. All samples are from Malaysia and were found only on the banana cultivar 'Pisang mas' raising the possibility that it is specific to that host.

Phylogenetic analysis of 15 isolates of *M. eumusae* from Asia and Africa identified two genotypes that were found across these continents.

Analysis of 92 samples of *M. fijiensis* from around the world identified five major clades (Black 1 to Black 5) that varied by one to two nucleotides. Seven branches formed around these clades - the maximum variation between any two strains was 6 nt.

The IGS region was also sequenced in a number of samples to further investigate the relationship between isolates. This region varied in size from 3.5 kb in *M. eumusae*, 4-5.5kb in *M. fijiensis* to 6kb in *M. musicola*. Homology between the three species was observed at the beginning and end of the IGS region but not in the central region. Thus, the IGS sequence alignment within each species was good, although the alignment between the species was very poor. This resulted in numerous large gap insertions for the central region of the alignment. The IGS data linked previous Australian outbreaks of BLSD to the major genotype found in the Torres Strait. The recent outbreak in Tully 2001 was shown to consist of two genotypes, one that was found in the Torres Strait, Papua New Guinea (PNG) and the Philippines. No exact homologue was identified for the second genotype though it is similar to an isolate from PNG.

Overall, *M. musicola* strains showed the most variation in the ITS region whereas *M. fijiensis* strains showed the largest variation in the IGS region.

Genetic structure of *Mycosphaerella fijiensis* populations at the continental scale

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The genetic structure of populations of *Mycosphaerella fijiensis* was studied by sampling 13 populations from banana plantations in Latin America and Caribbean region and Africa. The isolates were analysed using CAPS (Cleaved Amplified Polymorphic Sequences) and microsatellite molecular markers. The highest levels of genetic diversity in the Latin America and Caribbean region were found in the populations from Costa Rica and Honduras and the lowest levels were observed in the Caribbean populations. In Africa, genetic diversity levels were similar between countries, with the exception of Côte d'Ivoire and the Comoros where the levels were approximately half. A high level of genetic diversity and random mating populations were maintained even at the plant scale. Overall estimates of F_{st} were 0.19 and 0.30 in African and Latin America-Caribbean regions respectively, indicating a high and significant ($p<0.001$) level of genetic differentiation. A high and significant level of genetic differentiation was detected between most pairs of populations within each region.

The founder effects observed in the genetic structure of *M. fijiensis* in the African and Latin American and Caribbean regions is consistent with a stochastic spread of the disease at continental and country scales rather than a steady advance of an epidemic front. Genetic and epidemiological evidence support single-step invasions of the disease in the different regions of the world through the movement of infected plant materials rather than ascopore dispersal between continents. Expansion of the range of *M. fijiensis* populations within continents may result either from limited ascospore dispersal over a few hundred kilometers or from the movement of infected plant material. Although the relative importance of these two dispersal processes could not be determined, some evidence of movement of infected plant material strongly supports the improvement of quarantine measures to limit the risk of disease introduction in new areas and exchanges between existing populations from different countries. The sustainability of disease resistance management strategies will first depend on the effectiveness of quarantine measures. Disease management should also try to limit gene flow between pathogen populations via natural dispersal. Parameters corresponding to these evolutionary and epidemiological processes should therefore be estimated accurately.

Molecular diagnosis of black leaf streak disease in Australia

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Endemic to many parts of the world, including Papua New Guinea and Torres Strait Islands, black leaf streak disease, caused by the fungus *Mycosphaerella fijiensis*, is a major quarantine threat to Australian bananas. There have been nine incursions of the disease in Far North Queensland since 1981, with the most recent and serious one in 2001 at Tully, the first one in a commercial banana production area. The disease has been successfully eradicated on each occasion by the Queensland Department of Primary Industry through a vigilant programme of surveillance and deleafing.

One of the keys to successful eradication is early detection. Experienced plant pathologists are generally able to diagnose leaf spots using microscopic fungal structures for identification. In the absence of such structures, they rely on molecular based diagnostics. The polymerase chain reaction (PCR) has been used since 1998 to assist the diagnosis of leaf spot diseases in banana. An improved DNA-based assay developed by the CRC for Tropical Plant Protection (CRCTPP), capable of distinguishing Sigatoka disease and black leaf streak disease, was used extensively in Tully and is currently used for material collected during disease surveys. To ensure the robustness of the assay, we sequenced the internal transcribed spacer regions (ITS) of over seventy Australian and overseas isolates of *Mycosphaerella musicola* and *Mycosphaerella fijiensis*, as well as other *Mycosphaerella* species found to infect banana, including *Mycosphaerella eumusae,* the pathogen causing Eumusae leaf spot disease (ELSD). We have also included several other banana foliar pathogens and other Eucalyptus-infecting *Mycosphaerella* species to assist with the development of this diagnostic.

Alignments of the *M. fijiensis*, *M. musicola* and *M. eumusae* sequences revealed an extremely high degree of homology (approximately 98%) between these three species, with only small regions of variability. Interestingly, some of the conserved variations between *M. fijiensis* and *M. musicola* were also found in *M. eumusae*. This finding complicates the design of diagnostic primers for gel-based assays to efficiently differentiate them. This is an important issue as ELSD has been identified as an emerging disease of concern to our industry due to its presence in Southeast Asia.

The CRCTPP has now developed a new DNA-assay based on real-time, quantitative PCR that is even more sensitive than the current method. Quantitative fluorescent real-time PCR, in particular the TaqMan® probe system, offers increased specificity that cannot be achieved in gel based assays without post PCR processing. Consequently we designed primer and Taqman® probe sets specific for Sigatoka disease and black leaf streak disease. The primer and probes sets have been optimized and validated using our extensive collection banana leaf spot pathogens to ensure that our latest test is not only extremely sensitive but also extremely reliable. The assays have been duplexed with an internal control assay that detects a banana pectate lyase gene. This new test enables rapid and early diagnosis of *Mycosphaerella* leaf spot diseases in banana.

Evaluation of diploid and tetraploid hybrids against Fusarium wilt

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In Brazil, Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense*, is present in all the banana growing areas and is responsible for severe yield losses in the cultivar 'Maçã' (AAB) and, to a lower extent, in 'Prata' (AAB), 'Pacovan' (AAB) and 'Prata anã' (AAB). The disease is controlled by growing resistant cultivars, mainly those of the Cavendish subgroup. The breakdown of resistance reported in several banana growing areas, points to the necessity of generating resistant banana hybrids. The objective of this work was to evaluate the reaction of improved diploid (AA) and tetraploid (AAAB) hybrids to inoculation with *F. oxysporum* f. sp. *cubense*, in order to select resistant diploid hybrids to be used as male parental in banana breeding programs, as well as tetraploid hybrids to be delivered as resistant varieties.

The evaluation site was infested by growing 'Maçã'. To promote an even distribution of the inoculum, the area was ploughed and ripped before planting. To assure high inoculum levels, each plant under evaluation was surrounded by four susceptible plants in a completely randomized design with 10 replicates. The diploid hybrids 0116-01, 1304-04, 1304-06, 1318-01, 4223-06 and 5119-01 from Embrapa and SH3263 from the *Fundación Hondureña de Investigación Agrícola*, and the tetraploid hybrids PC42-01, PV03-44, PV42-53, PV42-68, PV42-81, PV42-85, PV42-129, PV42-142 , PV42-143, ST12-31, ST42-08 and YB42-21 from Embrapa and FHIA-03 and SH-3640 were evaluated. The susceptible cultivars 'Maçã' and 'Figue pomme naine' were used as control. Disease incidence was evaluated at harvest by cross sectioning the rhizome and rating vascular discoloration based on a scale varying from zero (no discoloration), to five (total discoloration).

The analysis of variance showed highly significant differences (P=0.01) between diploid and tetraploid hybrids in the first and second production cycles. According to the Scott & Knott test, all diploid hybrids were resistant to Fusarium wilt (disease severity up to 0.6). The tetraploids PV42-53, PV42-68, PV42-81, PV42-142, PV42-143, ST42-08, ST12-31, PV03-44, FHIA-03 and SH36-40 expressed resistance to Fusarium wilt in the first cycle, and PV42-53, PV42-68, PV42-81, PV42-85, PV42-142, PV42-143, ST42-08, ST12-31, PV03-44, FHIA-03 and SH-3640 in the second cycle. Despite showing internal symptoms similar to 'Maçã', YB42-21 did not collapse, as did the susceptible control, and yielded good quality bunches. Intermediate reactions were observed in PV42-129 and PC42-01.

Resistance to Fusarium wilt of somaclonal variants

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Somaclonal variants of the local cultivars PKZ, PKF, PKB, PKM and PKS were tested for resistance to Fusarium wilt. Tissue culture plants were planted in soil heavily infested with *Fusarium oxysporum* f. sp. c*ubemse* race 4 at Kiepersol and in non-infested soil at Burgershall and 'Williams' was used as susceptible control. Only 4.2% of the PKZ plants had died 3 years after planting, compared with 60% in the control treatment. PKF and PKS showed some tolerance, but not enough for release to banana producers. PKZ yielded 36.5 tons/ha in the mother-plant crop, which was the highest yield observed, and 48 tons/ha in the first ratoon crop. The interval from planting to harvest 55 days longer for PKZ than 'Grande naine' in the mother-plant crop and 3 days longer in the first ratoon crop. The average yield for PKZ in non-infested soil and in infested soil were similar. PKZ has been sent to Australia to be evaluated for its tolerance to Fusarium wilt race 1, 2 and 4 and black leaf streak disease. If PKZ is also tolerant to black leaf streak disease, it could be a winner in all tropical banana countries where this disease is a problem.

Resistance to Fusarium wilt race 4 in populations of *Musa acuminata* ssp. *malaccensis*

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Fusarium wilt of banana is endemic in the centre of origin of edible and wild bananas, which includes Indochina, Thailand and Peninsular Malaysia. These are also recognized as centres of diversity of the pathogen *Fusarium oxysporum* f.sp. c*ubense* (Foc). The presence of highly resistant wild banana populations in this region is probably the result of natural selection expressed through prolonged association between the fungus and its seed-propagated host.

Screening of suckers from a population of *Musa acuminata* ssp. *malaccensis* (AA) in a field infested with Foc Race 4 for over two years confirmed the resistance of this population. The progenies derived from an *in vitro* zygotic culture[1] were found to segregate for resistance in greenhouse assays. Survival of seedling lots varied between 13% and 82%, presumably due to genetic differences resulting from out-crossing in the field. More than 300 seedlings from 4 populations were screened. For three of the populations, chi-square analysis supported a 3:1 resistance:susceptible ratio, suggesting that resistance was controlled by a single dominant factor. Screening of plants with different RAPD primers revealed bands that were specific for either resistant or susceptible plants. SCAR markers are being developed from these bands. Controlled crosses were made between susceptible and resistant plants and F1 progeny were regenerated from seeds using the *in vitro* zygotic technique. These plants are being assessed for segregation for resistance in the greenhouse and a clonal population is being assessed in the field with the RAPD and SCAR markers.

1. Asif M.J., C. Mak and R.Y. Othman. 2001. *In vitro* zygotic embryo culture of a wild *Musa acuminata ssp. malaccensis,* and factors affecting the germination and seedling growth. Plant Cell, Tissue and Organ Culture 67(3):267-270.

In vitro screening for resistance to *Radopholus similis*

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Two *in vitro* experiments, using 'Grand naine' as the susceptible reference, were conducted to establish their reliability as methods to screen for resistance. In the first experiment, four Philippines cultivars that had displayed some resistance *in vivo* were screened for resistance to a Ugandan population of *Radopholus similis*. Four-week-old plantlets in rooting medium were inoculated with 25 gravid females and incubated at 28°C under a 16–hour photoperiod. Eight weeks after inoculation, the root fresh weight and number of nematodes in the roots and medium were determined. The cultivars 'Senorita' and 'Pamoti on' displayed the highest levels of resistance while the number of nematodes on 'Matavia' and 'Pisang lemak manis' were not significantly different from those on 'Grand naine'.

The second experiment was designed to examine the expression of resistance of 'Pisang jari buaya' and 'Yangambi km 5' over time. Plantlets were inoculated and incubated in the same manner as in the first experiment. Roots were harvested 2, 4, 8, 12 and 16 weeks after inoculation and the number of nematodes in the roots and medium were counted. Histological analyses of the roots were also performed. Significant interactions were found between time and cultivar for most of the parameters. At week 8, 'Pisang jari buaya' had a significantly lower number of nematodes than 'Grand naine', while the numbers on 'Yangambi' was not different from those on the other two cultivars. At week 16, the total numbers of nematodes on each cultivar were similar.

Staining root sections with Toluidine Blue O revealed the presence of lignified/suberized cells walls in the central cylinder and endodermis of 'Pisang jari buaya' and, to a lesser extent, 'Yangambi'. Such thickenings were not observed on 'Grand naine'. Occurrence of phenolic cells in the stele and cortex were observed in the three cultivars, but the expected accumulation of such cells in 'Yangambi' was not detected. Lignification of cell walls of endodermal and stellar tissues needs to be reevaluated as a mechanism of resistance.

IMTP-3 in Malaysia: preliminary results of reaction to Fusarium wilt

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Screening for resistance to Fusarium wilt disease was conducted on various hybrids and somaclones (FHIA-18, FHIA-21, FHIA-25, SH-3640, GCTCV-106, GCTCV-215, GCTCV-247, CRBP-39, BITA-2, BITA-3). The local reference clones were the dessert cultivars 'Mas', 'Berangan', 'Rastali', 'Novaria' and 'Montel' and the cooking clones 'Tanduk', 'Nangka', 'Raja', 'Abu nipah' and 'Awak'. Planting was carried out on a Fusarium-infested site at MARDI, Serdang, using a completely randomized design with 20 replications/clone.

Pseudostem splitting, one of the early diagnostic symptoms of Fusarium wilt, was observed as early as 2 months after planting on 'Montel' and 'Berangan Intan'. At five months, 10% of FHIA-21 hybrids showed pseudostem splitting while 10% of BITA-3 died. The percentage of stem splitting in the reference local clones varied between 10 and 20% and 'Montel' also recorded 10% death. The percentage of pseudostem splitting in 'Gros Michel', 'Williams', 'Bluggoe' and 'Yangambi km 5', the standard reference clones, were respectively 70%, 10%, 0% and 20%.

Status of banana nematodes in India and their management

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Nematodes constitute one of the major limiting factors to banana production causing extensive root damage. A total of 132 species of nematodes belonging to 54 genera are reported to be associated with the rhizosphere of banana. The most destructive and widely distributed nematode is the burrowing nematode, *Radopholus similis,* which can reduce yield by up to 41%[1]. This nematode was reported in almost all the banana growing areas of the country ,including isolated areas like the Andaman and Lakshadweep islands. The infested plants exhibit stunted growth, premature defoliation and carry small bunches and fruits. Small cuticular sunken lesions appear on young cord roots and extensive reddish brown lesions are observed in the cortex when it is cut longitudinally. The nematodes cause decay and death of distal cells and the plants are prone to toppling over in wet and windy weather because of inadequate anchorage.

The root-lesion nematode, *Pratylenchus coffeae,* is considered to be next to the burrowing nematode in importance. Losses of up to 44% have been reported[2]. The symptoms produced by this nematode are similar to those of *R. similis* and often its damage is attributed to the latter. The other economically important nematode pests of banana are the spiral nematodes, *Helicotylenchus multicinctus* and *H. dihystera,* the root-knot nematodes, *Meloidogyne incognita* and *M. javanica*, the cyst nematode*, Heterodera oryzicola,* and the reniform nematode, *Rotylenchulus reniformis*. The spiral nematodes infect the roots and corm and produce large brownish and black lesions that cause the plants to topple over. Losses of up to 100% have been reported in heavily infested 'Giant Cavendish' fields after the first year[3]. *M. incognita* reduced yield by 31% in Tamil Nadu[4].

Nematodes can be controlled by using integrated approaches such as cultural, physical, crop rotation, biocontrol agents, organic amendments and nematicides. Using nematode resistant cultivars and clean suckers have had success in the field. Methods for managing nematodes will be discussed.

1. Nair K.K.R. 1979. Studies on the chemical control of banana nematodes. Agric. Res. J. Kerala 17:232-235.

2. Sundararaju P and I. Cannayane. 2003. Evaluation of different biopesticides against major nematode pathogens infesting banana cv. Nendran. Pp. 66-67 *in* National Symposium on Organic Farming in Horticulture for Sustainable Production. August 29-30, 2003, CISH, Lucknow.

3. Zem A.C and E.J. Alves. 1981. Observations on losses caused by nematodes on banana (*Musa acuminata* cv. Nanicao). Boletin de pesquisa, Centro Nacional de Pesquisa de Mandioca e Fruticultura, Embrapa No. 6, 10p.

4. Jonathan E.I. and G. Rajendran. 2000. Assessment of avoidable yield loss in banana due to root-knot nematode, *Meloidogyne incognita*. Indian J. Nematol. 30:162-164.

Current issues in plantain research at IITA with emphasis on nematodes

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Plantain is a demanding crop in terms of soil fertility, and often the first crop to be introduced following fallow or bush/forest clearance. Production is afflicted by numerous problems such as nematodes, weevils, diseases and declining soil fertility, which exacerbates pest and disease problems. These constraints cause plant loss, undermine yield per plant, increase length of cropping cycle and reduce plantation longevity. Improvement of plantain production in the region would be beneficial from a number of different angles. The International Institute of Tropical Agriculture (IITA) is currently involved in a programme to improve plantain production in the region, through the development of pest and disease resistant material, use of healthy planting material and improved agronomic practices.

While most banana researchers recognize the importance of nematodes attacking the banana root system, the problem is often overlooked because the symptoms are less obvious than those caused by the banana weevil, leaf spot diseases and wilts. Farmers often direct management attention to visually apparent constraints and neglect root health. The nematode problem comprises a complex of several species, which rarely occur in isolation, but more as a species community. The population dynamics and interspecies competitiveness is relatively undetermined, while pathogenicity of individual species and species mixtures requires further investigation. Such information is relevant for the improvement of plantain through nematode resistance breeding.

While the identification and use of resistance against nematodes is a primary aim at IITA, the intention is not to depend on it exclusively. Rather, resistance (and/or tolerance) is being sought with the aim of harnessing it within an integrated pest management ‘basket’ of options towards sustainable improvement of plantain productivity in West Africa, which include using and promoting clean planting material, beneficial microbial agents and organic mulch.

In order to achieve the development of sustainable recommendations that will be adopted by farmers, complementary strategic research is conducted to support the more downstream developmental aspects of the programme. Such strategic research includes assessment of the importance and population dynamics of individual nematode species, and the importance of constraints in relation to each other.

Endophytes from wild bananas and their potential in suppressing Fusarium wilt

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The potential of endophytes in inducing some form of resistance to Fusarium wilt in susceptible banana seedlings was investigated. Endophytes were isolated from roots of wild banana plants, and screened *in vitro* for their antagonistic properties towards *Fusarium oxysporum* f. sp. *cubense* race 4 (FocR4). Two fungal (UPM31P1 and UPM31F4) and three bacterial (UPM13B8, UPM14B1 and UPM39B3) endophytes were selected as potential biocontrol agents, based on their percentage of inhibition of radial growth (PIRG) values of 58%, 65%, 52%, 52% and 63%, respectively. These endophytes were also effective host colonizers and can be re-isolated from the roots, leaves and pseudostem, 24 hours after inoculation.

UPM31P1 and UPM39B3 significantly improved plant growth and induced host resistance. When tested on seedlings inoculated with FocR4, UPM31P1 and UPM39B3 and a mixture of UPM13B8, UPM14B1 and UPM39B3 suppressed the development of Fusarium wilt, when compared to the control. Seedlings inoculated with UPM31P1 and UPM39B3 also grew better. However, to maximize control, a multiple inoculation is suggested as the population of endophytes recovered from the host system decreases over time.

Use of plant growth-promoting rhizobacteria to enhance tolerance to Fusarium wilt

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*Department of [1]Land Management, [2]Crop Science and [3]Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia*The introduction of beneficial microorganisms for biological control of soil borne plant pathogens and enhancement of plant growth has an immense potential in agriculture. The anti-fungal abilities of these beneficial microbes have been known since the 1930s and there have been extensive efforts to use them for plant disease control. However, they have only recently been used commercially. Some of the bacteria associated with roots, known as plant growth-promoting rhizobacteria (PGPR), and enhance plant growth.

Fusarium oxysporum f.sp. *cubense* (Foc) is a soil-borne plant disease that causes severe yield losses in several crops including bananas. Current research has shown the potential of PGPR to control the disease caused by Foc. Two PGBR, *Azosprillum* sp and *Bacillus* sp (UPMB10), reduced by 60-70% the *in vitro* radial growth of an isolate of Foc race 4 (FocR4). In liquid culture, UPMB10 produced swellings and distorted hyphae of FocR4. Conidium germination was also reduced in the presence of UPMB10. Greenhouse experiments were subsequently done to evaluate the effect of PGPR on root development, nutrient and water uptake and growth of bananas, and to determine the effect of PGPR on the development of Fusarium wilt. The initial results suggest that UPMB10 has potential as a biocontrol agent against Fusarium wilt of banana.

The International Banana Action Plan

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In many countries, the intensive use of agro-chemicals in banana production represents an environmental threat. The annual input of pesticides to control nematodes and fungi is among the highest in agricultural crops. For example, even in very well designed and maintained plantations, more than 30% of the total production costs are devoted to the control of the foliar pathogen *Mycosphaerella fijiensis*, the causal agent of black leaf streak disease. In a year, approximately 2.5 billion US$ are spent on fungicide applications. An additional problem is the nematode *Radopholus similis*. Although control costs are much lower, the damage can be extensive and the environmental impact of the highly toxic nematocides is considered to be substantial.

Recently, the International *Mycosphaerella* Genomics Consortium was set up under the coordination of INIBAP with the aim of exchanging and generating genomic and genetic information on *Mycosphaerella* species, using the wheat-*Mycosphaerella* pathosystem as a model to structure research, including programmes for comparative genomics. This has already resulted in international collaborative projects aiming at mapping the *M. fijiensis* genome and isolating the mating type genes in a range of *Mycosphaerella* pathogens of banana. Meanwhile *in vitro* and *in vivo* EST libraries of *M. fijiensis* were constructed and sequenced, and proposals for sequencing the *Mycosphaerella* pathogens of wheat and banana have been submitted to international sequencing organizations. This body of genetic and genomic data will provide insights into the genes controlling pathogenicity and host resistance.

Encouraged by the international drive to resolve the environmental issues in banana production, Wageningen University and Research Centre initiated the International Banana Action Plan. Through an integrated approach, its aim is to reduce pesticide input in banana production by at least 50% over the coming decade. The plan will focus on the control of *Mycosphaerella* diseases and *Radopholus* pests in banana production and will include programmes addressing fundamental as well as applied questions. These will include programs on 1) Pathogen genomics and genetics, 2) Genetics and breeding for resistance, 3) Epidemiology and population genetics, 4) Soil Science, 5) Biological control, 6) Precision farming and disease management strategies and 7) Extension and social impact programmes. We believe that an intensified international and integrated approach will eventually lead to the development of innovative control strategies for important biotic and abiotic threats, which in turn will lead to the adoption of environmentally friendly banana production systems for both large-scale and smallholder plantations. Our vision, strategy and activities for an International Banana Action Plan will be presented and discussed.

Potential of fungal endophytes in nematode management

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Plant parasitic nematodes cause substantial production losses to both banana and plantain throughout the world. Strategies to manage nematode pests of *Musa* include the use of resistance, healthy planting material and improved agronomic practices. However, in many situations, additional options are required to improve and sustain nematode management and consequently *Musa* production. In East Africa, nematode management using mutualistic endophyte fungi has been researched at the International Institute of Tropical Agriculture (IITA), Uganda, in collaboration with the Uganda National Agricultural Research Organisation, Makerere University, Pretoria University in South Africa and the University of Bonn in Germany.

Endophytes occur commonly in plants as asymptomatic parasites, which cause no damage to the host, but can suppress or repel pest damage. In Uganda, a wide diversity of endophytic fungi has been isolated from banana tissue. The most frequently isolated fungal genus from subterranean banana tissue has been *Fusarium,* followed by *Acremonium*. Avirulent strains of *Fusarium oxysporum,* the most frequenyly isolated species*,* are the focus of attention in the current study.

Isolates of *F. oxysporum* from the corm and roots have been screened for *in vitro* activity against the burrowing nematode *(Radopholus similis*). Results have shown high levels of nematicidal activity of the culture filtrates for a number of isolates *in vitro* against *R. similis*. Certain isolates have also suppressed nematode reproduction by 30-40% given following inoculation into banana plantlets. Nematode control effects have been observed for up to 7 months after inoculation. Further assessment is required regarding the effect of fungal endophytes against diseases and pests other than *R. similis*. Although the mechanisms that are responsible for nematode suppression are not known yet, it is believed that effective control requires colonization of and persistence within the plant. Studies are currently investigating the persistence of inoculated isolates in banana and the extent to which different isolates colonize banana tissue.

Fungal endophytes could provide a significant additional tool for nematode management that should have its greatest impact when applied to tissue culture plantlets. With wide acceptance of tissue culture planting material among African farmers, endophytes may significantly contribute to effective and durable biological pest and disease control in *Musa*.

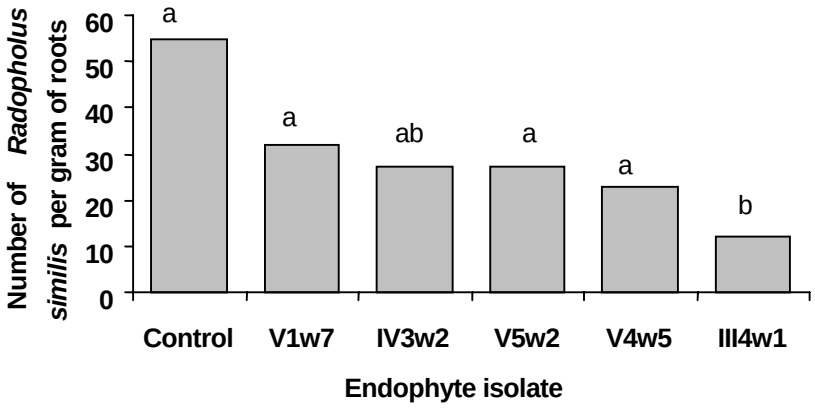

Figure 1. Radopholus similis *multiplication 12 weeks after inoculation of 1000 nematodes on 42-week-old tissue culture banana plants cv. 'Valery' (AAA).*

Use of arbuscular mycorrhizal fungi to control nematodes

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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that biotrophically colonize the root cortex of host plants and develop an extraradical mycelium in the rhizosphere. These fungi help the plant to acquire water and mineral nutrients from the soil in exchange for carbon as an energy source. In addition, AMF increase the ability of a plant to overcome abiotic and biotic stress and to reduce colonization by soil-borne pathogens. Early mycorrhizal colonization of *Musa* varieties result in improved plant growth, although the magnitude of this response, expressed as the relative mycorrhizal dependency (RMD), depend on the plant variety. Mycorrhizal dependency has been defined as the degree to which a plant is dependent on mycorrhizae to produce maximum growth or yield at a given level of soil fertility. Besides improving root growth, mycorrhizal colonization also changes root architecture.

Banana varieties with a proportionally high primary root weight had a medium to high RMD and the root system was significantly more branched. In the banana varieties with a proportionally high root weight of secondary and tertiary roots, the AMF had no influence on the branching of the root system. These varieties had a low RMD. Early root colonization by AMF suppressed population build-up of the burrowing nematode *Radopholus similis* and the root-lesion nematode *Pratylenchus coffeae* in roots of all *Musa* varieties tested while the effect on development of root necrosis was variable. In the mycorrhized plants the nematode population density was on average 67% lower compared to the densities in the non-mycorrhized plants. In most *Musa* varieties, neither *R. similis* nor *P. coffeae* affect the percentage of root colonization by the fungi. Based on our results, implementation of early mycorrhizal inoculation at the nursery level can represent an alternative method for the management of banana nematodes when banana plantlets are transplanted to nematode-infested soils. However, it is crucial to identify the factors contributing to the expression of the biocontrol ability of AMF if appropriate inoculation or management techniques are going to be developed. Therefore, a histological study combined with bio-assay studies on the root exudates and root extracts were the first steps taken to reveal the mechanisms responsible for this horizontal resistance against nematodes. Preliminary results will be presented.

Efficiency of fallow and hot water treatment in reducing nematodes in Cameroon

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Small-scale farmers in the Central Province of Cameroon traditionally plant their plantains in secondary or primary forest following slash-and-burn preparation of the land. Due to increasing population pressure however, fallow periods are declining and crop pest populations are increasing. This study reports on the effectiveness of hot water treatment in reducing plant-parasitic nematodes on two plantain varieties (*Musa* spp., AAB, French, cv. 'Essong' and AAB, False Horn, cv. 'Ebang') in a short and long fallow system. Fertilizer was applied as a fourth factor. Sampling was done at 15 and 21 months after planting (MAP), respectively, coinciding with the flowering/harvest of the first crop cycle and 6 months thereafter.

Initially (15 MAP), a hot water treatment on the plantain suckers before planting significantly reduced nematode numbers in both fallow systems and increased the overall root system health, as indicated by the non-damaged root index. However 21 MAP, the population increases of plant-parasitic nematodes in short fallowed plots outweighed these benefits, making the choice of field location the dominant factor. The question remains how long farmers will be able to make this choice. The importance of the development of adapted sustainable technologies for plantain production is discussed.

Effect of mulching on the yield 'Agbagba' inoculated with nematodes in southeastern Nigeria

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Production reduction caused by nematodes in plantain was evaluated at Onne, near Port Harcourt in southeastern Nigeria, 10 m above sea level. The commonly grown cultivar 'Agbagba' (AAB) was either inoculated or uninoculated in heavily mulched and non-mulched management regimes. The nematode population consisted of the following species: *Helicotylenchus dihystera, Helicotylenchus multicinctus*, *Hoplolaimus pararobustus*, *Meloidogyne* spp. and *Radopholus similis*. Influence of the different treatments was evaluated for the first crop cycle and mulching was observed to have the greatest influence on production. The uninoculated heavily mulched plots produced 8.1 t/ha compared to 3.6 t/ha in the uninoculated non-mulched plots. Plant parasitic nematodes were responsible for the heavy damage inflicted on the root and corm of plantain. Plants that were not inoculated with plant parasitic nematodes were generally taller and had thicker pseudostem than the inoculated ones. The nematode-induced losses were a result of a reduction in bunch weight and an increase in plant toppling. The yield reduction by nematodes in heavily mulched plants was 46% compared to 54% in the non mulched plants. Higher incidence of toppling (23%) was observed in mulched plants, compared to 16% in the non-mulched ones.

Biocontrol of lesion nematodes

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A field trial was conducted in a farmer's field at Coimbatore to study the effect of biocontrol agents on nematodes. The results revealed that the application of *Pseudomonas fluorescens* at 20g/plant was associated with the highest bunch length (95 cm), bunch weight (24 kg), number of hands per bunch (10) and of fingers per bunch (176), compared to the other treatments. The increase in yield parameters ranged from 59 to 110% compared to the control and the populations of *R. similis, P. coffeae* and *H. multicinctus* were reduced by 48, 46 and 44%, respectively. Soil application of *T. viride* and carbofuran 3G were the next best treatment followed by the application of VAM fungus, *B. subtilis* and *P. lilacinus*. The untreated control recorded the lowest bunch weight (15 kg). Application of carbofuran 3G was found effective in reducing nematode population during the early growth stage up to three months. Thereafter its efficacy was reduced and was comparable to the most effective biocontrol agent, whereas *P. fluorescens* maintained its efficacy throughout the crop cycle.

Integrated pest management of banana stem weevil *Odoiporus longicollis*

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Odoiporus longicollis (Coleoptera Curculionidae) is a key pest of banana in India and mainland Asia. Infestation starts in 5-month-old plants. Early symptoms of attack include the presence of small pin sized holes on the stem, fiber extrusions at the base of petioles having adult weevils and jelly exudation from holes on the pseudostem. Chemical pesticides are expensive and beyond the reach of small-scale farmers. Moreover, they are hazardous to health and environment. This paper presents integrated pest management technologies developed at the National Research Centre for Banana.

Sanitary measures are imperative. Dried and old leaves must be removed to detect early symptoms of weevil infestation. The practice of leaving banana pseudostem stumps (matocking) in endemic areas should be stopped as adult weevils survive in old banana mats.

Adult weevils were found to be naturally infected with fungi such as *Metarrhizium anisopliae* and *Scopulariopsis brevicaulis* and entomopathogenic fungi such as *Beauveria bassiana*, *Beauveria brongniartii*. Some of these fungi were evaluated against the banana stem weevil under laboratory conditions. *B. bassiana* seemed to be effective in controlling the stem weevil.

Host plant resistance is a component of an IPM strategy. Two hundred accessions were screened under laboratory conditions and some accessions such as 'Bhimko l' (BB), 'Athiakol' (BB), 'Elavazhai' (BB), 'Saapkal' (AAB), 'Dudhsagar' (AAA) and 'Pisang jari buaya' (AA) were found to be resistant.

Plant products were also extracted using organic solvents and screened against adult weevils under laboratory conditions. Among them *Vitex negundo* (leaf extract), *Terminalia chebula* (seed extract), *Acoras calamas* (rhizome extract) caused some mortality. These extracts could be sprayed over the pseudostem to prevent oviposition of the stem weevil. Isolated stem weevil pheromone components evaluated under field conditions also seem to have potential.

Efficacy of pseudostem and pheromone traps against *Cosmopolites sordidus* (Germar) in South Africa

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The dominant insect pest of bananas in South Africa is the banana weevil, *Cosmopolites sordidus*, (Coleoptera: Curculionidae), which is also an important insect pest of bananas worldwide. In developing an integrated pest management strategy for *C. sordidus* in South Africa, different trapping methods were compared in field trials for their efficacy in capturing adults during autumn, spring and summer along the south coast of KwaZulu-Natal.

Pseudostem traps, two pheromone lures (individually suspended above a pitfall trap) and control traps (pitfall traps without pheromone) were evaluated for their efficacy in collecting adults over five week periods. During the autumn trial series, two additional treatments were included: corm traps and pseudostem traps treated with a synthetic pyrethroid insecticide. Pitfall traps were filled with a mixture of ethylene glycol and water to reduce evaporation and lower the surface tension of the solution in which the attracted beetles drowned. The pseudostem and corm traps were covered with mulch to delay dessication and decomposition. Pseudostem and corm traps were replaced once a week, when the samples per trap were collected and counted. Adults were dissected, sexed by examining internal genitalia and the number of eggs and oocyte presence were noted. A randomized block design was used for all trials, with replicates orientated perpendicular to the moisture gradient in the field and the sea-land breeze.

The two pheromone pitfall traps collected significantly more adult beetles than the control and pseudostem traps during autumn, spring and summer. Pheromone trap efficacy did, however, decline significantly in the fall. The pseudostem traps were significantly more effective than the control traps, regardless of the season; whereas the insecticide treated traps were generally not significantly more effective than the control traps. Seasonal indices of increase in relation to pseudostem trap catches were calculated for pheromone traps. Significant differences in adult sex ratio were observed within seasons between the plant material traps and the pheromone ones. The females attracted to pheromone traps and plant material traps showed differences in fecundity within a season. The relatively low maintenance and high efficacy of pheromone traps shows promise for the development of a monitoring and possible control strategy against the banana weevil in South Africa.

Biological control of the banana weevil in Africa

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The banana weevil *Cosmopolites sordidus* is the most important insect pest of bananas and plantains in Africa. The larvae enter the corm, reducing nutrient uptake and weakening the stability of the plant. Attack in newly planted banana stands can lead to crop failure. In established fields, weevil damage can result in reduced bunch weights, mat die-out and shortened stand life. Damage and yield losses tend to increase with time.

The weevil's biology makes its control difficult. It is characterized by nocturnal activity, long lifespan, limited mobility, low fecundity and population growth. The adults are free-living and attracted to their hosts by plant volatiles. Males produce an aggregation pheromone that is attractive to both sexes. Eggs are laid in the corm or lower pseudostem. The immature stages are within the host plant.

This paper reviews biological control attempts in Africa against the banana weevil. These have included the use of exotic natural enemies (classical biological control), endemic natural enemies, secondary host associations and microbial control (entomopathogens and endophytes). Microbial control agents may require multiple applications as biopesticides and thus entail repeated application costs on the part of the farmer.

The banana weevil evolved in Asia, the center of origin of *Musa,* and as such it is an exotic pest in Africa. Early searches in southeast Asia revealed several generalist predators. Releases of these predators in Africa and elsewhere met with little success. Recent searches for parasitoids in Indonesia were also unsuccessful. Further searches for natural enemies in India are merited. Attempts to use egg parasitoids of another weevil through secondary host association were likewise unsuccessful. Research in East Africa on endemic natural enemies suggests most local predators have limited promise. However, the ants *Pheidole* spp. and *Odontomachus troglodytes* will attack immature stages within host plant tissue and are currently being evaluated in the field.

Research protocols for developing microbial control of banana weevil with the entomopathogen *Beauveria bassiana* entail isolation, characterization, screening and pathogenicity testing of candidate strains, field-testing, and evaluation of mass production and delivery systems. Various strains of *B. bassiana* have caused 50-100% mortality in two weeks in the laboratory. Some isolates have displayed good growth and spore production on locally available substrates. Field delivery of possible delivery systems of *B. bassiana* showed that the application of the fungi with planting material, pseudostem traps or soil around the base of the mat can be used to infect the banana weevil in the field and reduce damage caused to the plant. These studies have demonstrated that *B. bassiana* shows promise as a microbial control agent against the weevil. Further research is being undertaken to integrate *B. bassiana* with other banana weevil IPM options, such as the use of semiochemical attractants, and the development of economically viable delivery systems that will overcome the problems associated with field fungal efficacy, persistence and disease transmission.

Biocontrol of Fusarium wilt: A review and an evaluation

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Fusarium wilt is one of the most destructive diseases of banana[1]. Its current and future impact has received considerable attention and recently fueled concerns about the crop's extinction. Although the disease will certainly not exterminate banana, Fusarium is nonetheless a serious problem and difficult to manage[2]. Effective fungicides are not available, resistant breeding parents are scarce, pathogen-free soils in which susceptible clones could be grown are uncommon, and disease-suppressive soils are rare.

Biocontrol has been investigated as an alternative approach for managing Fusarium. Diverse microbes have been tested, including arbuscular mycorrhizal fungi, *Bacillus* spp., fluorescent pseudomonads, nonpathogenic endophytes, and *Trichoderma* spp. They have usually been used in *in vitro* and greenhouse assays that assessed direct inhibition of the pathogen, disease reduction in small plants grown in pots, and biochemical traits of the pathogen and host. Field studies have been infrequent, and generally provide scant support for this approach. With one possible exception, biocontrol has not been successful in production situations.

For several reasons, Fusarium is a more difficult target for biocontrol than most other plant diseases. Since banana is usually grown over several years for multiple cycles, disease control measures must be effective for a long period. Even an 18% annual loss, the best reported result for a biocontrol treatment in a refereed journal[3], would result in a compounded loss of 63% after 5 years. The complex soil environment in which the pathogen resides complicates protection of infection courts, and its vascular location after infection occurs isolates it from the vast majority of biocontrol agents. These difficulties will be discussed during comparisons of biocontrol studies on Fusarium and other pathosystems.

1. Ploetz R.C. 2003. Pesticide Outlook 14:62-64.

2. Ploetz R.C. and K.G. Pegg. 2000. Fusarium wilt. Pp. 143-159 *in* Diseases of Banana, Abacá and Enset (D.R. Jones, ed.) CABI Publishing, Wallingford, UK.

3. Saravanan T. *et al.* 2003. Crop Protection 22:1117-1123.

Actinomycete Streptomyces g10 as a potential biocontrol agent against Fusarium wilt

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Antagonistic bacteria isolated from Fusarium wilt suppressive soils, fluorescent *pseudomonas* and *Trichoderma viride* have been used to control Fusarium wilt *in vitro*. So far, there has been only one attempt to evaluate the efficacy of antagonistic actinomycetes in field experiments. *Streptomyces* spp., well known as a source for antibiotics and lytic enzymes, have been studied extensively as potential biological control agents against fungal phytopathogens. *Streptomyces lydicus* WYEC108 was selected based on its strong *in vitro* activity against *Pythium ultimum*.

Streptomyces sp. strain g10 isolated from a sand sample of a coastal sandbar in Port Dickson, Malaysia, demonstrated strong activity against a range of phytopathogenic fungi. Assigned to the *Streptomyces violaceusniger* clade, strain g10 produced extracellular antifungal metabolites that strongly inhibited spore germination and hyphal development of Foc race 4 in plate assays. The biocontrol ability of strain g10 against Foc infection of 4-week-old tissue culture plantlets of 'Novaria' was evaluated by adding a suspension (10^8 cfu/ml) of strain g10 to the soil before planting in the greenhouse. The colonizing ability of the strain was also evaluated. Strain g10 significantly ($p<0.05$) reduced disease severity in plantlets inoculated with 10^4 spores/ml of Foc race 4. Disease severity index for leaf and rhizome discoloration were reduced by 47% and 53%, compared to control plantlets. Population of strain g10 in the rhizosphere more than doubled at the end of the third week.

Potential of endophytic bacteria against banana bunchy top virus in India

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Among the biotic and abiotic stresses affecting bananas, bunchy top virus is responsible for important yield losses. In recent years, the utilization of endophytic bacteria is emerging as a potential tool for the management of many plant diseases. We evaluated 40 isolates of endophytic bacteria collected on corms for their capacity to control bunchy top virus.

To assess the efficacy of the selected isolates against banana bunchy top virus a pot experiment was conducted with cultivar 'Robusta' (AAA). A formulation containing 2.8 x 10^8 cfu of bacteria was prepared by mixing bacterial cells with sterilized talc powder. Suckers were inoculated with the endophytic bacteria (10 g/L). The virus sources were maintained in the greenhouse and the aphids were allowed to feed on them. The plants treated with endophytic bacteria were inoculated with the viruliferous aphids (*Pentalonia nigronervosa)*. The plants were tested for the presence of virus using ELISA and the virus titre was compared among the plants treated with endophytic bacteria.

The activity of defense related proteins, such as peroxidase, polyphenol oxidase, phenylalanine ammonialyase and chitinase, increased up to 7 days after inoculation, regardless of the treatment and generally declined thereafter. Gel electrophoresis of plants extracts showed 6 peroxidase and polyphenol oxidase isoforms in the plants treated with endophytic bacteria and inoculated with *P. nigronervosa* compared to 3 in the control plants. The enzyme extracts from endophytic bacteria treated plants infested with aphid showed six chitinase isoforms compared to two in the control plants infested with aphids. The plants treated with endophytic bacteria seem more resistant to the bunchy top virus and the aphid vector. Endophytic bacteria could be used to control economically important plant diseases in horticultural crops.

Effect of *Pseudomonas fluorescens* bioformulation on plant growth promotion and postharvest anthracnose incidence in banana under field conditions in southern India

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In field experiments conducted at Coimbatore, Tamil Nadu, India, talc-based bioformulations of *Pseudomonas fluorescens* (strains Pf1 and FP7), *Bacillus subtilis* (Bs1), *Saccharomyces cerevisiae* (Sc1) and *Phaffia* sp. were applied to banana plants 'Robusta' at 3, 5 and 7 months of planting at 10g per plant. Bunch spray was also given at 0.5% of the bioformulation during last hand emergence and then two sprays at 30-day intervals. The influence of soil application and bunch spray alone and the combined effect of soil application and bunch spray on certain plant growth parameters and bunch characters and postharvest anthracnose incidence were studied.

Soil application of the bioformulation with FP7 amended with chitin and FP7 alone increased plant height by 34% and 24% respectively compared to control. The bioformulation of FP7 and Sc1 amended with and without chitin significantly increased pseudostem girth. Combined soil and bunch treatment and soil application alone did not affect bunch length. Soil application combined with bunch spray of FP7 bioformulation amended with chitin increased bunch length by 18%. Slight increase in the number of hands was noticed by soil application alone with FP7, Sc1 and chitin amended bioformulation of Pf1, FP7 and Sc1. Bunch sprays alone could impart a marginal increase in the number of hands in the treatments involving PF1 and FP7 bioformulations. Combined soil and bunch treatment with FP7 amended with chitin led to a 38% increase in bunch length. Yield increase was more pronounced (28%) in treatment involving combined soil and bunch application of FP7 amended with chitin.

Reduction in postharvest anthracnose incidence at room temperature was maximum (87%) in the treatment involving combined soil and bunch application of FP7 bioformulation amended with chitin followed by FP7 alone, Sc1 amended with chitin (78% reduction each) and Sc1 alone (75% reduction). In cold storage (15°C), FP7 bioformulation amended with chitin and FP7 alone reduced anthracnose severity by up to 89% and 55% respectively, followed by chitin amended formulation of Bs1 which reduced the disease by 44%.

Session 2

Plant protection

Posters

P31 - Screening for resistance to nematodes at Kannara, India

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In Kerala, southern India, surveys revealed the presence of the following nematode genera *Radopholus, Pratylenchus, Helicotylenchus, Meloidogyne* and *Criconemoides*[1] and the cyst nematode, *Heterodera oryzicola*[2]. The Banana Research Station in Kannara has one of the richest field genebanks in the country. Its 256 accessions are being screened to identify sources of resistance/tolerance to major pests and diseases.

Field screening conducted at harvest revealed that the most resistant or tolerant cultivars belonged to the AAB and AB groups. The AAB cultivars 'Thekkanthulladen', 'Malbhog', 'Padathi', 'Mottapoovan', 'Charakali', 'Kalibow', 'Amrithapani' and 'Karibale' recorded an average root necrosis between 0 and 5%. 'Nendran' (AAB), the most popular variety in Kerala, was average. The AB cultivars 'Agniswar' and 'Poomkannan kadali' had an average root necrosis between 2% and 3%. The AAA cultivars, such as 'Grande naine', 'Sapumal anamalu', 'Chakkarakeli', 'Moris' and 'Monsmarie' were susceptible, with an average root necrosis between 50% and 75%. However, the AAA cultivars 'Namkanika', 'Karivazha' and 'Nakitemp' were more resistant. The ABB cultivars were equally distributed between the resistant, average and susceptible groups. 'Sambranimonthan', 'Paloor' and 'Vellapalayankodan' were some of the resistant cultivars in this genomic group. The results at flowering were similar, except that the average root necrosis percentages were slightly lower than those at harvest.

1. Charles J.S.K., T.S. Venkitesan and K. Pushkaran. 1988. Nematodes attacking banana and their control. A technical bulletin. Directorate of Extension, KAU.

2. Charles J.S.K. and T.S. Venkitesan. 1990. Banana cyst nematode *Heterodera oryzicola* Rao and Jayaprakash, 1978. Poster demonstrated at the Poster session of the Zoological Society of London Symposium, 3-4 May, 1990.

P32 - Susceptibility of *Musa* germplasm to the banana stem weevil, *Odoiporus longicollis*

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Rapid screening methods to evaluate the behaviour of M*usa* accessions against the banana stem weevil, *Odoiporus longicollis,* were developed at the National Research Centre for Banana (NRCB). One hundred accessions from the NRCB genebank were screened under laboratory conditions.

Adult weevils were allowed to feed on 30 cm long leaf sheaths for ten days and then removed. The leaf sheaths were examined for damage. A transparent plastic sheet was placed over the leaf sheath to trace the damaged area, which was later quantified using graph paper.

The proportion of the leaf area damaged was arc-sine transformed, statistically analysed and classified as follows: no damage (immune) 1-5% damaged (highly resistant), 6-10% (resistant), 11-15% (moderately resistant), 16-20% (moderately susceptible), 21-25% (susceptible), over 25% (highly susceptible).

Diploids, triploids, tetraploids, commercial cultivars and exotic accessions exhibited significantly different percentages of damage. None of the accessions were immune. Accessions such as ‘Nendran’, ‘Midnoli’, H-201, FHIA-01, ‘Pisang Ceylan’, ‘Ramban’, ‘Namarai’, ‘Kere’, ‘Pagar banana’, ‘Monthan’ and ‘Karpooravalli’ were the most susceptible to the banana stem weevil. Native diploids such as ‘Bhimkol’, ‘Athiakol’, ‘Elavazhai’ and ‘Saapkol’ and exotic accessions such as ‘Pisang lilin’ and ‘Pisang jari buya’ were found resistant to *O. longicollis.* These accessions could be used in breeding programmes.

P33 - On-station and on-farm evaluation of improved hybrids and popular local cultivars to rehabilitate banana industry in the Philippines

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In the last 15 years, *Musa* breeding programmes have produced a number of high yielding and disease resistant hybrids of banana and plantains, some of which are considered to be suitable for smallholder production in the Philippines. In collaboration with the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development and several state universities in Luzon, the aim of the project is to introduce improved hybrids to small-scale farmers. A total of 32 197 tissue culture plants of five improved hybrids and two popular local cultivars were distributed to five state universities and colleges in Luzon, where demonstration trials were set up, and to farmers in four agro-ecological zones. These hybrids are being evaluated as part of a plan to rehabilitate the local banana industry, which has been devastated by the banana bunchy top virus and other diseases. Preliminary data indicate that some of the hybrids show resistance to the virus.

P34 – Survey of nematodes in Quezon province, Philippines

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Survey of population, prevalence and distribution of parasitic nematodes associated with banana cultivars were conducted in 19 barangays and seven towns of Quezon province, Philippines. Root damage assessment expressed as percentage of dead roots, percentage of root necrosis and nematode counts per 10 grams of roots were obtained. Five nematode genera were found associated with banana cultivars: *Radopholus similis, Pratylenchus, Helicotylenchus, Rotylenchus reniformis* and *Meloidogyne incognita*. Root damage assessment data showed a range of 0 to 45% of dead roots and a percentage necrosis of 2.5-62.0%.

P35 - Reaction of FHIA hybrids and landraces to Fusarium wilt

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Fusarium wilt caused by *Fusarium oxysporum f. sp. cubense* (*Foc*) is one of the most important diseases of banana. Foc populations in Cuba belong to race 1 and 2 and to the vegetative compatibility groups (VCG) 01210, 0124, 0124/0125 and 0128. Inoculations were carried out with isolates VCG 0124, VCG 0128 and VCG 01210. The inoculum was reproduced on twice-sterilized sorghum seeds inoculated with a Foc conidial suspension and incubated at room temperature. Fifteen grams of infested seeds were applied at the bottom of holes before planting tissue culture plantlets (Table 1) in a completely randomized design with five replications. As expected, 'Manzano' and 'Gros Michel' reacted as cultivars susceptible to race 1 and 'Burro criollo' as susceptible to race 2. FHIA-03 was susceptible to the isolates in VCG 0124, but not to the other isolates. Despite their close relatedness, VCG 0128 killed all 'Pelipita' plants, whereas VCG 0124 had no effect. These results indicate the limitations of the current classification and the need for a better understanding of pathogenic specialization.

Table 1. Reaction of cultivars to inoculation with different vegetative compatibility groups (VCG) of *Fusarium oxysporum f. sp. cubense*

Cultivars	Proportion of diseased plants (%)			Mean severity*		
	VCG 01210	VCG 0124	VCG 0128	VCG 01210	VCG 0124	VCG 0128
Manzano criollo	60	0	0	4.6	1.0	1.0
Gros Michel	100	0	0	5.0	1.0	1.0
Burro criollo		60	40	1.0	4.0	3.8
Pelipita	0	0	100	1.0	1.0	5.0
Pisang awak	25	0	33.3	2.4	4.3	4.5
Musa acuminata 2	66.6	33.3	33.3	1.8	1.3	4.3
Pisang lilin	25	0	25	4.7	2.6	4.3
Calcutta 4	0	33.3	25	4.5	2.3	2.2
Pisang jari buaya	20	40	20	1.6	3.6	3.2
Paka	66.6	0	0	4.3	5.0	5.0
Yangambi km 5	100	33.3	33.3	5.0	3.3	4.0
FHIA-02	0	0	0	0.0	0.0	0.0
FHIA-03	20	60	0	1.8	2.4	3.6
FHIA-04	0	0	0	0.0	0.0	0.0
FHIA-18	20	0	0	1.8	0.0	1.6
FHIA-21	0	20	0	0.0	2.6	1.0

* Scale from 0 (healthy) to 5 (dead).

P36 - Evaluation of banana weevil damage assessment methods on East Africa highland cooking banana

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The banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an important pest on bananas and plantains. Population build-up is slow and weevil problems become increasingly important in successive crop cycles. Yield loss results from plant loss (death, snapping, toppling), mat disappearance (failure to sucker) and reduced bunch size. Damage assessment requires destructive sampling and is most often done on the corm periphery or corm cross sections of recently harvested plants. A wide range of damage assessment methods exist and there are no agreed upon assessment protocols. In this context, it is critical to know what types of damage best reflect weevil pest status through their relationships with yield loss.

Multiple damage assessment parameters were employed in two long duration yield loss trials using 'Atwalira', an East Africa highland banana (AAA) and a cultivar screening trial in Uganda. Parameters included two estimates of peripheral damage on pared corms and estimates of damage to the central cylinder and cortex (plus a derived total damage score) observed in cross sections. In the first two trials, estimated yield losses to banana weevil exceeded 40% in latter cycles.

Damage to the central cylinder had a greater effect on plant size and yield loss than damage to the cortex or corm periphery. In some cases, a combined assessment of damage to the central cylinder and cortex showed a better relationship with yield loss than an assessment of the central cylinder alone. Correlation analysis showed weak to modest relationships between damage to the corm periphery and damage to the central cylinder. Thus, damage to the corm periphery (less labour intensive to assess) is not a strong predictor of the more important damage to the central cylinder. Therefore, banana weevil damage assessment should be made for the central cylinder and cortex.

P37 - Characterization of fungal endophytes as possible biological control agents against *Fusarium oxysporum* f.sp. *cubense*

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The soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (Foc) causes Fusarium wilt, one of the most destructive diseases of bananas[1]. No control option other than resistant plants currently exists for the management of this disease. Biological control provides an opportunity to control Fusarium wilt in an environmentally friendly way[2]. It is known that the incidence of Fusarium wilt can be reduced in banana fields where so-called "suppressive soils" occur. Suppressiveness is due to the actions of various biotic and abiotic factors. Non-pathogenic *F. oxysporum* is one of the biotic factors that contribute to reducing Fusarium wilt in suppressive soils[3]. Non-pathogenic isolates of *F. oxysporum* that live inside banana roots (endophytes) have also shown to increase host resistance to Foc[4]. The objective of this study was to determine whether non-pathogenic *F. oxysporum* endophytes found in the roots of bananas in suppressive soils could contribute to Fusarium wilt control.

The diversity of the endophytic populations was determined, as well as the latter's ability to protect banana plants against infection by Foc. Samples were taken from banana roots grown in three suppressive soils in Kiepersol, South Africa. Endophytic *F. oxysporum* was isolated from the Komada[5] medium and morphologically identified. The isolates were further characterised by means of PCR-RFLP analysis of the intergenic spacer region (IGS) of the rRNA operon[6]. Five enzymes were used, *Msp*I, *Scrf*I, *Hindf*I, *Hae*III and *Rsa*I, and the *F. oxysporum* isolates were divided into haplotypes. Representatives of each haplotype were chosen to determine their virulence to small banana plants. Non-pathogenic isolates were evaluated for their potential to suppress Foc *in vitro* and in the greenhouse. PCR-RFLP analysis proved to be a sensitive method to detect diversity among *F. oxysporum* strains and to categorize groups of closely related strains at the intraspecific level. The haplotypes were able to distinguish between pathogenic and non-pathogenic isolates of *F. oxysporum*. The non-pathogenic isolates did not control Foc *in vitro,* suggesting that the mechanism of control is not antibiosis. Representatives of *F. oxysporum* obtained by PCR-RFLP analysis will be evaluated for their ability to control Foc in the greenhouse.

1. Ploetz R.C. 2000. Panama disease: A Classic and Destructive Disease of Banana. [WWWdocument – 18/03/2003]. http://www.planthealthprogress.org/current/management/bananapanama/top.htm

2. Wardlaw C.W. 1961. Banana diseases including plantains and abaca. Longmans. London. Pp. 188-209.

3. Louvet J., C. Alabouvette & F. Rouxel. 1981. Microbiological suppressiveness of some soils to Fusarium wilts Pp. 260-275 *in* Fusarium: Diseases, Biology, and Taxonomy (P.E. Nelson, T.A. Toussoun, & R.J. Cook, eds). The Pennsylvania State University Press. University Park and London.

4. Gerlach K.S., S. Bentley, E.A.B. Aitken, N. Moore & K.G. Pegg. 1998. Do non-pathogenic *F. oxysporum* suppress Fusarium wilt of banana? The 7th International Congress of Plant Pathology, Edinburgh, Scotland.

5. Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Protec. Res 8:114-124.

6. Edel V., C. Steinberg, I. Avelange, G. Laguerre & C. Alabouvette. 1995. Comparison of three molecular methods for the charaterization of *Fusarium oxysporum* strains. Phytopathology 85:579-585.

P38 - Effectiveness of traps to control the pseudostem borer of banana

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The pseudostem borer, *Odoiporus longicollis* (Oliv.), is one of the most widespread and devastating pest of bananas in South India. Symptoms often go unnoticed by farmers, consequently leading to important reductions in yield. Effective chemical control strategies are available against this pest, which are being used indiscriminately by farmers leading to environmental pollution and health hazards. Hence, alternative methods like bait trapping were considered.

Several authors have reported the use of traps made of cut pseudostems to attract the pseudostem borer of banana.[1,2]. One study found that the best control method was trapping adults with pseudostem traps that were big enough for oviposition but too thin for larval development[3]. The effectiveness of cut pseudostem pieces, along with other locally available and commonly used insect bait materials, such as toddy, dried fish and ripe banana pulp was tested at the Banana Research Station in Kannara. The pseudostem pieces attracted the highest number of weevils (43 weevils/week) while the other materials showed negligible or no catch at all.

The pseudostem traps required changing every week in summer due to drying. However, during the rainy season, the traps remained fresh up to three weeks. It was also observed that the number of weevils attracted increased as the bait decayed. However, by the fourth week, when decaying was almost complete, the numbers caught decreased, indicating that the weevils did not prefer completely rotten pseudostems.

The placement of traps was also examined. It was observed that length, orientation and height had an effect on the attractiveness of the traps. Pseudostem traps 0.5 to 0.75 m long placed on the ground attracted the highest number of weevils. Pseudostem traps were effective only when the pest population in the field was medium to high.

1. Kung K.S. 1964. Ecological studies on banana stem borer weevil, *Odoiporus longicollis* Oliv. J. Agric. for Taiwan. 11: 137-160

2. Padmanabhan B., R. Rajeswari and S. Sathiamoorthy. 2002. Banana pseudostem trapping technique for management of banana weevils (Coleoptera: Curculioniidae). Global Conference on Banana and Plantain, 28-31 October 2002, Bangalore, India, 187 pp.

3. Hoffmann W.E. 1932. Observations on a weevil injurious to banana. Hong Kong Nat. 4(1):48-54

P39 - Effect of endophytic bacteria on Fusarium wilt

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Fusarium wilt, caused by *Fusarium oxysporum* f.sp*. cubense* (E.F.Smith) Snyder and Hans, is a major constraint to the production of bananas in many tropical and subtropical parts of the world. The pathogen penetrates the plant root system and eventually occludes the vascular vessels causing great loss to the farmers in terms of productivity and yield. Although chemicals are available for the management of the disease, they have demonstrated limited efficacy and are linked to health and environmental problems. The use of bio control agents is a safer method for managing the disease.

We focused on the use of endophytic bacteria isolated from bananas to induce systemic resistance against Fusarium wilt. Forty bacteria were isolated from corms and tested for their effect on growth and vigour index in rice. Among these isolates EPB 5 and EPB 22 were found to increase the vigour index of rice seedlings significantly when compared to control. EPB 22 and EPB 5 also showed antagonistic activity against *Fusarium oxysporum* f.sp*. cubense in vitro*.

A pot culture experiment was conducted with 'Rasthali' (AAB). The results revealed that EPB 22 + Pf1 was significantly superior in reducing the discoloration of side roots*.* The strain mixture recorded only 15% discoloration of side roots, compared to control plants in which 85% was observed. Some of the defense related enzymes were studied and it was observed that peroxidase, polyphenoloxidase, phenylalanine ammonialyase and phenol levels increased up to seven days after inoculation and declined thereafter. However, in the control plants there was no induction of these defense enzymes suggesting the involvement of these proteins in inducing systemic resistance against Fusarium wilt. Based on this, a field experiment was carried out to assess the efficacy of endophytic bacteria*.* EPB 22 + Pf1 significantly reduced the disease, compared to control. Thus, endophytic bacteria may provide an alternative means for managing Fusarium wilt.

P40 – Efficacy of biocontrol agents against *Pratylenchus coffeae* and *Meloidogyne incognita*

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The root-lesion nematode, *Pratylenchus coffeae,* and the root- knot nematode *Meloidogyne incognita,* are important nematode pests of banana and are usually controlled by using synthetic nematicides. An alternative is using biocontrol agents. Eight biocontrol agents (*Trichoderma harzianum, Verticillium chlamydosporium*, *V. lecanii, Paecilomyces lilacinus, Arthrobotrys oligospora, A. cladodes, Pseudomonas fluorescens* and *Bacillus subtili*), the organic amendment Neem cake and the nematicide Carbofuran 3G were tested on 'Rasthali' grown in pots under field conditions. All treatments reduced nematode populations and enhanced plant growth, compared to control plants. *P. fluorescens, P. lilacinus, V. chlamydosporium, B. subtilis and V. lecanii* were associated with highest plant growth and significant reduction in nematode populations. Neem cake and Carbofuran 3G had similar effects on the plants.

P41 - Evaluation of new nematicides

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The root-lesion nematode, *Pratylenchus coffeae,* and the root-knot nematode, *Meloidogyne incognita,* are important pests of banana. New nematicides, Rugby 10G, Caldan 4G (granules), Caldan 50SP (powder) and a plant growth promoter, Biovita, were compared with Carbofuran 3G on 'Nendran' (AAB) grown in pots under a net. All treatments significantly reduced the nematode population and increased plant growth, including fruit yield, compared to untreated control plants. The highest bunch weights (20 and 18 kg) were recorded on plants treated with 10 g and 15 g of Rugby 10G per plant, 50 g of Carbofuran 3 G per plant and 30 ml of Biovita per plant applied in two applications. The lowest bunch weight (10 kg) was recorded in the untreated control plants. The cost benefit ratio worked out to be much cheaper with Rugby 10 G and Caldan 4G than with Carbofuran 3G and other treatments.

P42 - Efficacy of a biofungicide based on metabolites of *Pseudomonas aeruginosa* to control crown rot

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Crown rot, caused by *Fusarium pallidoroseum* and *Colletotrichum musae* and a complex of *Fusarium* species, are the most important postharvest diseases of banana and are usually controlled by chemical fungicides. *Pseudomonas* spp. have been used as biocontrol agents[1]. The application of purified siderophores as bacteriostatic or fungistatic agents has raised interest[2]. We investigated the efficacy of gluticid, a bio-fungicide based on metabolites of *Pseudomonas aeruginosa* isolate Pss obtained by liquid fermentation[3].

Two trials were carried out using four replicates of eight clusters each of 'Granded naine' hands. The crowns were washed in running water treated with the different products, left to dry and inoculated with a spore suspension (9 conidia/field) of 7-day-old cultures of *F. pallidoroseum* and *C. musae* conidia. The crowns were refrigerated at 14ºC for 21 days. Assessment of disease development was carried out a 3, 7 and 14 days after treatment using a nine degree severity scale (from 0=healthy crown to 9=rotting affecting peduncles and fruit). The severity of the disease (proportion of the crown's surface that is rotted) was calculated.

Symptoms appeared after three days. Fourteen days after inoculation, gluticid showed an inhibitory effect on crown rot that was comparable with the one of prochloraz (Figure 1). In the second trial, all treatments significantly inhibited the development of crown rot. Disease severity after 14 days using thiabendazole at 400 µg/ml, prochloraz at 400 µg/ml, gluticid at 200 µg/ml and gluticid at 300 µg/ml was respectively 20.3%, 22.1%, 28.4% and 18.6%, compared to 48.7% in the control treatment. There was no difference between prochloraz, thiabendazol and gluticid at 300 µg/ml. These results show the potential of this fungicide for the postharvest treatment of organic bananas.

1. Wilson M. 1997. J. Ind. Microbiol. Biotechnol. 19:188-191.

2. Díaz de Villegas M., P.E. Villa and A. Frías. 2003. Revista Latinoamericana de Microbiología 44:112-117.

3. Villa P., A. Frías, I. Gutiérrez, M. Stefanova and I. Alfonso. 2001. Boletín de la Sociedad Cubana del Suelo. Ciudad de la Habana, Nov. 2001. 67pp.

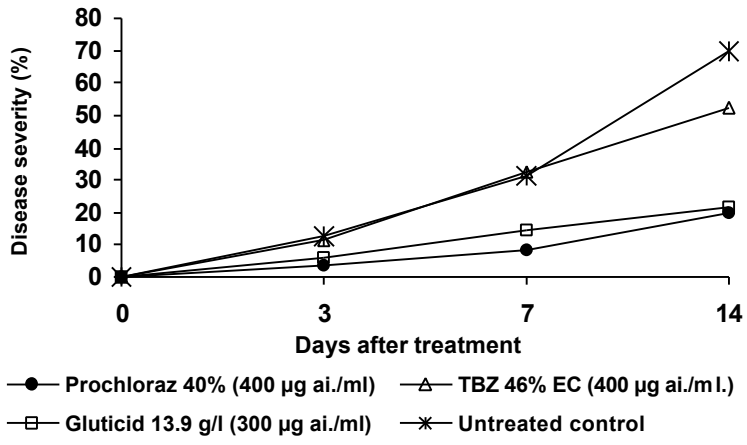

Figure 1. *Effects of various products on the disease severity (percentage of rotted surface) of crown rot.*

P43 - Effect of biocontrol agents on a *Fusarium* wilt-nematode complex in cv. 'Rasthali'

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Fungal and bacterial antagonists against Fusarium wilt and nematodes were tested on 'Rasthali' (AAB) plants grown in pots and inoculated with the nematodes *Pratylenchus coffeae* and *Meloidogyne incognita* and the fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). Ten grams per pot of *Trichoderma harzianum*, *Paecilomyces lilacinus* and *Bacillus subtilis* and 25 g per pot of VAM were applied. A random block design with 9 replicates was used. After 2, 4 and 6 months, the plants were pulled out and wilt incidence, root galling index, root lesion index, plant height, collar girth, number of leaves, leaf area, number of roots, root length and root weight were recorded.

The application of biocontrol agents significantly reduced Fusarium wilt incidence, nematode populations and root lesion and root knot indices in inoculated plants. The highest reduction in Fusarium wilt (56%) was observed when *T. harzianum* was applied to plants inoculated with *P. coffeae* and Foc. It was followed by *B. subtilis* (28%), *Glomus mosseae* (22%) and *P. fluorescens* (22%). In the case of the *M. incognita*-Fusarium wilt complex, the maximum reduction (50%) was observed with *T. harzianum,* followed by *B. subtilis* (35%) and *G. mosseae* (22%). When all the three pathogens were present, a 50% reduction was observed with *T. harzianum,* followed by *B.subtilis* (22%) and *G. mosseae* and *P. lilacinus* (13%). Populations of *P. coffeae* and *M. incognita* were reduced by 78% and 82% respectively with *T. harzianum*.

P44 - Non-pathogenic Fusarium isolates as biocontrol agents

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This study was aimed at developing control methods using native antagonistic microorganisms. Two fungal (*Fusarium* spp.) isolates from the rhizosphere of 'Pisang awak' (ABB) and 'Robusta' (AAA) and one endophytic *Fusarium* sp. isolate from the 'Rasthali' (Silk, AAB) inhibited the mycelial growth of Foc race- 1 *in vitro*. The isolates did not cause any symptoms of wilt disease when used to root-dip inoculate tissue culture plants of 'Rasthali'. When used in different ways (soil application of sand maize inoculum, root-dip inoculation and a combination of both methods), the rhizosphere isolates from 'Pisang awak' and the endophytes from 'Rasthali' completely arrested the development of Fusarium wilt in race 1-inoculated 'Rasthali' in pots. Biochemical analyses indicated that these isolates increased the level of total phenols and peroxidase activity in the plants.

P45 - Contribution to the development of a biological control method against crown rot disease

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Crown rot is an important banana post-harvest disease infecting the pad of cut hands. The disease spreads rapidly during fruit ripening and contaminates the pedicel and ultimately the pulp. The disease is caused by a complex of fungi (occasionally bacteria) with one main pathogen: *Colletotrichum musae*. The aim of this study was to evaluate the antagonistic activity of two yeast strains against the parasitic complex in relation with their concentrations and their incubation period.

The antagonistic activity of *Pichia anomala* strain K and *Candida oleophila* strain O, applied at three concentrations (10^6, 10^7, 10^8 cfu/ml), have been evaluated on *Colletotrichum musae* (10^3 conidia/ml), *Fusarium moniliforme* (10^4 conidia/ml), *Cephalosporium sp.* (10^4 conidia/ml) or on a parasitic complex formed by the association of these three fungi. One hundred µl of the pathogen(s) were deposited on the crown of 4 'Grande naine' (AAA) hands. After 15 minutes, the crowns were soaked in a suspension of strain K or strain O for 10 seconds. Before the evaluation of the pathogen(s) progression into the crown, bananas were incubated for 13 days under conditions similar to exportation. Antagonistic effects have been observed on *C. musae*, *F. moniliforme* and the complex. Among the various treatments, the application of strain O at 10^8 cfu/ml showed the highest protective level (56 %). This level was higher than those observed against the fungi separately inoculated.

The influence of incubation period between strain O (10^8 ufc/ml) and complex inoculation has also been studied. Strain O was applied 24h before the complex, but also 15' or 3h after its inoculation. The highest protective level was observed when strain O was applied 24h before the complex. Finally, a correlation between symptom severity and protective level of strain O has been highlighted. When the severity of the disease increased, protection by the yeast decreased. In conclusion, both strains have showed antagonistic effects but strain O was more effective than strain K.

P46 - Evaluation of cv. 'Angola' under different management systems

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The absence of integrated management practices in the banana plantations of the State of Acre, Brazil, favours the development of black leaf streak disease[1]. The present work was aimed at developing environmentally safe control methods, using agronomic practices that rely on the plant's physiology and the plantation's environment.

This study took place at Embrapa Acre's experimental field in October 1999. The cultivar 'Angola' was evaluated under various densities of planting (T1, 3.0 m x 3.0 m; T2, 3.0 m x 2.0 m; T3, 2.5 m x 2.0 m; T4, 4.0 m x 2.0 m x 1.5 m; T5, 2.0 m x 2.0 m; and T6, 3.0 m x 3.0 m under rubber trees). The T1 plots were weeded, a common practice among local producers, whereas the other plots were managed according to technical recommendations for banana growing.

The experimental design was a randomized block design, with five replicates and three plants per plot. The evaluations were conducted on the first ratoon crop. The number of functional leaves at flowering and harvest and the bunch weight were recorded. The severity of black leaf streak disease, using Gauhl's modification of Stover's severity scoring system[2,3], was evaluated monthly. The data were submitted to an analysis of variance and the averages compared by Scott & Knott's test.

There were significant differences between treatments regarding the number of functional leaves at flowering and harvest, the mean bunch weight and severity of black leaf streak disease (Table 1).

The plants cultivated at the 3.0 m x 3.0 m spacing under the shade of rubber trees (T6) presented the highest number of functional leaves at flowering and harvest, the highest average bunch weight and the lowest disease severity (Table 2). These results can be due to the low light level because of the rubber trees.

1. Cavalcante, M.J.B., T.M.S. Gondim, J.B. Ferreira, F.F. De Azevedo, Z.J.M. Cordeiro & A.P. Matos. 2001. Avaliação de sistema de manejo para convivência com a Sigatoka-negra no Estado do Acre. Fitopatologia Brasileira 26 (Suplemento):398.

2. Stover R.H. 1971. A proposed international scale for estimating intensity of banana leaf spot (*Mycosphaerella musicola)*. Tropical Agriculture, Trinidad, 48:185-196.

3. Gauhl F. 1994. Epidemiology and ecology of black Sigatoka (*Mycosphaerella fijiensis* Morelet) on plantain and banana (*Musa* spp) in Costa Rica, Central America. Thesis (translated by INIBAP). INIBAP, Montpellier, France. 120pp.

Table 1. Analysis of variance for the number of functional leaves at flowering (NFLF) and harvest (NFLH), the average bunch weight (BW) and the severity of black leaf streak disease (DS) in the cv. 'Angola' under different management systems in Rio Branco, Brazil.

Source of variation	Degrees of freedom	Mean Square			
		NFLF	NFLH	BW	DS
Replication	4	1.9442	0.3370	2.6530	73.3667
Treatment	5	22.5122**	2.4837**	4.0255**	846.9933**
Error	20	0.3695	0.0362	1.7334	84.6266

** Significant at 1% level.

Table 2. Impact of various management systems on the agronomic performance of cv. 'Angola'.

	Number of functional leaves		Bunch weight	Disease severity
	At flowering	At harvest		
T1	7 d	1.3 c	6.4 b	66.2 a
T2	9.2 b	1.3c	6.7 b	63.4 a
T3	9.1 b	1.5c	6.3 b	56.6 a
T4	8.2 c	1.2c	6.8 b	59.6 a
T5	9.3 b	1.9b	6.6 b	46.8 b
T6	13.3 a	3.1a	8.7 a	31.2 c

P47 - Presence of *Metamasius hemipterus* (L.) and *Cosmopolites sordidus* on plantain in Sur del Lago de Maracaibo, Venezuela

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The presence of *Metamasius hemipterus* and *Cosmopolites sordidus* (Coleoptera, Curculionidae) was sampled in seven plantain fields by using modified sandwich traps treated with Furadan. The traps were examined 24 hours after their placement, the number of insects per trap determined and the contents transferred to glass jars containing 70% alcohol for further studies and analysis. An analysis of variance was performed on the data. The results indicate that the populations of *M. hemipterus* and *C. sordidus* as well as the infection percentages were significant higher during certain seasons of the year, due to abrupt changes in rainfall and relative humidity in some of the sampled plots.

P48 - Interactions between nematodes and Fusarium wilt

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Banana is prone to attack by different pathogens such as fungi and nematodes. In India, Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), is widespread in almost all the banana growing regions of the country and cultivars such as 'Rasthali' and 'Virupakshi' are highly susceptible to this disease. Plant parasitic nematodes, such as *Pratylenchus coffeae* and *Meloidogyne incognita,* are also known to cause economic losses in India. Since these two nematodes are associated with *F. oxysporum* f. sp. *cubense,*[1] the present investigation was carried out to determine the individual and interactive effects on banana of *P. coffeae* and *M. incognita* with *F. oxysporum* f. sp. *cubense*.

A significant reduction in plant growth was recorded when plants were first inoculated with *P. coffeae* and *M. incognita* followed by Foc. The nematodes caused similar reductions in growth, whereas maximum plant growth was recorded in the uninoculated control plants. Higher nematode multiplication and root-lesion and root-knot indices were recorded in plants inoculated with nematodes alone than in plants treated first with nematodes, followed by Foc. Control plants and those inoculated with Foc alone did not yield any nematodes. Symptoms in the corm were significantly higher in plants inoculated with nematodes first followed by Foc than with any of the pathogens alone. External symptoms of Fusarium wilt appeared two months after inoculation in the nematode and Foc treatment, and four months in the case of Foc. Plants inoculated with either *P. coffeae* or *M. incognita* alone and uninoculated control plants did not produce any wilt symptom even six months after inoculation.

1. Thangavelu R., P. Sundararaju, S. Sathiamoorthy, T. Raghuchander, R. Velazhahan, S. Nakkeeran and A. Palaniswami. 2001. Status of Fusarium wilt of banana in India. Pp. 58-63 *in* Banana Fusarium Wilt Management: Towards Sustainable Cultivation. (A.B. Molina, N.H. Nikmasdek and K.W. Liew, eds). INIBAP-ASPNET, Los Baños, Laguna, Philippines.

P49 - Construction of *Mycosphaerella fijiensis* cDNA libraries from infected leaves of 'Calcutta 4' and 'Niyarma yik'

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Molecular studies of plant-pathogen interactions are necessary to identify genes that can be used in the genetic improvement of plants. The aim of this work was to construct cDNA libraries from infected leaves of cultivars that are resistant and susceptible to *Mycosphaerella fijiensis* Morelet.

Erlenmeyer flasks with 150 ml of V-8 modified medium were inoculated with the *M. fijiensis* isolate IBP 1 and incubated for 15 days on a rotary shaker at 120 rpm and 28°C. Mycelium was collected and ground in a Waring blender for 3 minutes (10 g of mycelium in 900 ml of sterile distilled water). The resulting suspension of mycelial fragments was adjusted to 10^6 cfu/ml and mixed with an aquose solution of gelatin 1%. Plants of 'Calcutta 4' and 'Niyarma yik' (20 cm high and with four functional leaves) were used for the inoculation. The lower surface of the first three open leaves of each plant was inoculated by using a brush. Leaves were allowed to dry for two hours and the humidity was maintained at 90% to 100% by continuously spraying water. After three days, the humidity was saturated during the night and suspended during the day.

RNA purification took place at different times of infection. Samples taken 25 and 34 days after infection were used for the isolation of total RNA. Purity and integrity of the samples were checked by spectrophotometry and in a gel. For the synthesis of first-strand cDNA the total RNA was treated with DNAse I. One microgram of total DNA-free RNA was subjected to RT-PCR with the oligo dT primer. First-strand cDNA synthesis was performed at 42°C for 1 hour followed by 15 minutes at 70°C. *M. fijiensis* cytochrome b gene forward and reverse primers were used to confirm the integrity of cDNA. Second-strand cDNA synthesis was performed by using the homopolymeric tailing method with dC-BamHI+dT-NotI primer combination. PCR products were successfully ligated into pGEM-T Easy vector. Four cDNA libraries of infected plants at different times of infection were obtained. Recombinant transformants were checked using colony PCR.

P50 - Identification of the fungus causing leaf speckle in West Malaysia

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Leaf speckle is a serious disease of banana in the tropics. It causes leaf necrosis in mature plants of 'Pisang berangan' and 'Mas' in Teluk Intan, Malaysia. A study was undertaken to elucidate the causal fungus with a view to aid disease diagnosis. Infected leaves show symptoms ranging from patchy yellow orange to purple brown discoloration. Coalescing lesions leads to leaf necrosis. Conidia from cellotape imprints of the fungi were mainly fusiform, aseptate or 1-septate. Pure cultures could not be obtained by direct plating. A single spore isolation method was required by scrapping conidia from diseased leaves into sterile water. One ml of conidia suspension was poured into a Petri dish containing 2% water agar amended with two antibiotics, streptomycin and tetracycline sulphate, at a concentration of 100 ppm. A single germinated conidium was picked and transferred onto potato dextrose agar plate. The colonies exhibited variations on culture media. Generally, they were light grey in colour with rounded margins, becoming darker at the center as they matured. Maximum vegetative growth was recorded on banana leaf extract agar, while maximum sporulation of up to an average of 7.25×10^6 per ml occurred on malt extract agar and potato dextrose agar after 4 weeks of incubation at ambient temperature. The study identified the fungus isolate from Teluk Intan to be synonymous with *Cladosporium musae* Mason.

P51 - Sensibility of *Mycosphaerella fijiensis* populations to triazole and strobilurin fungicides in Ecuador

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The increasing use of fungicides to control black leaf streak disease and the presence of high amount of inoculums in the banana areas of Los Ríos, Guayas and El Oro, prompted this study on the effect of triazole and strobilurin fungicides on disease development. The results are based on *in vitro* measurement of germination and the development of germination tubes of *Mycosphaerella fijiensis* ascospores in areas with and without fungicide applications.

The results with triazole fungicides showed a selection of a wider population with a resistance factor >1 and growth inhibition of germinating tubes below 50%. With strobilurin fungicides, the results showed appropriate sensibility levels with some specific exceptions. These results confirm the importance of developing disease control measures through the annual monitoring of fungal sensibility as an obligatory and legal tool to maintain fungicide effectiveness. Monitoring the sensibility of the commercial fungicides applied in banana plantations will obviously reduce application costs, improve environmental management and contribute to diminish inoculum increments, in particular of resistant populations.

P52 - Effect of paraffinic oil on *Mycosphaerella* leaf spot diseases

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Paraffinic oil (Banole®) is used against *Mycosphaerella* leaf spot diseases. A field trail was conducted with Robusta (AAA) an important commercial cultivar to evaluate its efficacy alone and in combination with half doses of some of the recommended fungicides. In total, 29 treatments were sprayed four times, every 25 days. Disease severity and youngest leaf spotted (YLS) were recorded 6 months after planting, at shooting and harvest.

The highest reduction in disease severity (79%) at harvest was observed using 5% oil + 0.05% companion. Generally, 5% oil + fungicides at half of the dose of recommended level was significantly better than the other treatments involving oil or fungicides sprayed separately. The highest increase in YLS (218%), compared to controls, was observed with 10% oil + 0.05% propiconazole. Oil alone (10%) increased YLS by 136%, compared to control. The greatest increase in the value of YLS was observed at harvest. Spraying oil alone increased yield, with the greatest increase observed with the 5% concentration. In generally, spraying oil in combination with fungicides at half of the recommended dose significantly increased yield compared to control (up to 29%).

P53 - Occurrence of peel and pulp splitting disorder in ‘Pisang mas’ during fruit maturation

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‘Pisang mas’ is susceptible to peel/pulp splitting disorder. Experienced farmers suggested that overripe fruits and late harvesting practice due to insufficient labour might encourage peel and pulp splitting disorder. A number of studies also state that fruit splitting is usually maturity related. As a pre-harvest disorder, fruit splitting might result from the fluctuation of soil moisture and relative humidity, dry wind, rain or heavy irrigation following a dry spell. Fluctuation of water supply in the early fruit growing season and excess uptake of water by fruit shortly before harvest might lead to cell rupture. To understand the nature of peel and pulp splitting, changes in peel and pulp moisture content, peel and pulp firmness, pulp to peel ratio during fruit growth and maturation of ‘Pisang mas’ in Raub (Pahang) were recorded.

Plants were tagged at flowering. Bunches were harvested 4, 5, 6, 7 and 8 weeks after flowering and the changes in the physicochemical characteristics determined. In both peel and pulp, changes in moisture content from week 4 to week 7 after flowering were not significantly different ($p<0.05$). Approaching week 8, all the parameters were significantly different ($p<0.05$) from the fruits assessed before. These changes coincided with the occurrence of peel and pulp splitting. Nine-week-old fruits of ‘Pisang mas’ should be ripe. In this study, 8-week-old fruits were significantly ($p<0.05$) less firm, indicating the onset of ripening. Pulp volume increased at higher rate than that of the peel, due to rapid pulp cell division. There was a significant increase ($p<0.05$) in the ratio of pulp to peel at weeks 4, 6 and 8. Pulp cell enlargement was at its peak by week 7 to week 8, where the ratio of pulp to peel was as high as 3.5. There is a possibility that the onset of splitting coincided with this period as well. During this time, expanding pulp might have met the stretching limit of the peel, exerting an internal force resulting in peel splitting and more extensively to the splitting of the pulp.

P54 - The prevalence of *Pratylenchus goodeyi* on bananas and plantains in mixed cropping systems of the Cameroon Highlands

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In Cameroon most subsistence farmers apply a mixed cropping system, resulting in a range of possible nematode host plants, each with a specific effect on the nematode community. Bananas and plantains (*Musa* spp.) are an important component of these cropping systems both as a source of cash income and as a staple food, and are found in almost every field. The Cameroon Highlands represent 10% of the total land area in Cameroon, but accommodate roughly 30% of the total population. In addition more than 25% of all plantains are produced in the region and more than 30% of all bananas. Nematodes have been identified as a major constraint to *Musa* production worldwide. In Africa, in production areas at higher elevation (lower temperature), *Pratylenchus goodeyi* is the dominant nematode species found associated with plantains, occurring almost exclusively in smallholder's fields. A survey was done throughout the Cameroon Highlands to identify the types of crop management practices, crop associations and pest awareness of small-scale farmers. In addition, samples were taken to examine the prevalence of *P. goodeyi* on bananas and plantains in the home garden and one field of each household visited. This study reports on the information gathered from more than 200 interviews and sampling visits in the West and North West Provinces of Cameroon.

P55 - Enhancing capacity for nematode management in small-scale banana cropping systems

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This five-year project aims to improve banana production at the smallholder level through the development of an alternative method for the management of nematodes in small-scale banana cropping systems, to strengthen research capacity at the College of Agriculture of the University of the Philippines Los Baños and to train Southeast Asian nematologists in banana nematology. The project activities will consist of a training-and-technology-transfer package, two research packages and two regional workshops. Information obtained on nematode host response and resistance in the Southeast Asian *Musa* germplasm will be made available to banana researchers and breeders via the *Musa* Germplasm Information System (MGIS).

P129 - Application of AFLP in genetic analysis of *Fusarium oxysporum* f.sp. *cubense*

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Fusarium oxysporum f.sp. *cubense* (*Foc*), the causal agent of Fusarium wilt, consists of several variants that are divided into three races and 21 vegetative compatibility groups (VCGs). Several DNA-based techniques have been used to analyse the worldwide population of *Foc* [1,2,3,4,5] . These techniques often yielded contradicting results and, therefore, a suitable molecular technique needs to be found to subdivide the worldwide population of *Foc* into subunits in order to determine genetic diversity and relationships between groups. In this study, the high-resolution genotyping method of amplified fragment length polymorphism (AFLP) was used to study the natural population of *Foc* at the genome-wide level.

The population selected included *Foc* isolates representing different VCGs and races, isolates of *F. oxysporum* f.sp. *melonis*, *F. oxysporum* f.sp. *lycopersici* and *F. oxysporum* f.sp. *dianthi*, the non-pathogenic biological control strain Fo-47, and *Fusarium circinatum*. Genomic DNA from all isolates was digested with *Eco* RI and *Mse* I restriction endunucleases and subsequently ligated with corresponding site-specific adapters. High-throughput AFLP analysis was achieved using five different infrared dye-labelled primer combinations and two-dye model 4200s LI-COR automated DNA analysers. AFLP digital images were scored using the SAGA^{MX} automated AFLP analysis software. An average of approximately 100 polymorphic loci was assayed simultaneously with each primer pair. AFLP fragments in the range of 50-750 bp were considered for analysis. Data files were generated based on the presence or absence of informative loci. For analysis, data of five primer combinations was combined. The data files were subjected to distance analysis, which included the use of neighbour-joining and a bootstrap of a 1000 replicates.

The distance analysis of AFLP data clearly showed two major groups. The first group contained isolates from all over the world, while the second group contained mostly isolates from Australasia. The distance tree also indicated that some lineages are polyphyletic and grouped closer to *F. oxysporum* of other *formae speciales* than to each other. These results support earlier suggestions that the banana wilt pathogen has independent evolutionary origins[5]. Great diversity among Asian isolates has also been found by AFLP analysis and supports the hypothesis that the pathogen has co-evolved with edible bananas and their diploid progenitors in Asia[6]. At least seven genotypic groups could be identified, which suggests that the current race structure in *Foc* should be reviewed. Our results indicate that AFLP is a powerful tool to perform detailed analysis of genetic diversity in *Foc*.

1. Boehm E.W.A., R.C. Ploetz and H.C. Kistler. 1994. Statistical analysis of electrophoretic karyotype variation among vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cubense.* Molecular Plant-Microbe Interactions 7:196-207.

2. Koenig R., R.C. Ploetz and H.C. Kistler. 1997. *Fusarium oxysporum* f.sp. *cubense* consists of a small number of divergent and globally distributed lineages. Phytopathology 87:915-923.

3. Bentley S., K.G. Pegg and J.L. Dale. 1995. Genetic variation among a worldwide collection of isolates of *Fusarium oxysporum* f.sp. *cubense* analysed by RAPD-PCR fingerprinting. Mycological Research 99:1378-1384.

4. Bentley S., K.G. Pegg, N.Y. Moore, R.D. Davis and I.W. Buddenhagen. 1998. Genetic variation among vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cubense* analysed by DNA fingerprinting. Phytopathology 88:1283-1293.

5. O'Donnell K., H.C. Kistler, E. Cigelnik and R.C. Ploetz. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Science, USA 95:2044-2049.

6. Stover R.H. 1962. Fusarial wilt (Panama disease) of bananas and other *Musa* species. Phytopathology paper No. 4 CMI 117pp.

P130 - Detection of geographical isolates of banana bunchy top virus of India

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Banana bunchy top virus causes severe losses in India. 'Virupakshi' (AAB), the most popular cultivar in Tamil Nadu has been almost destroyed by the disease. In this investigation, we compared DAC-ELISA, PCR and Nucleic Acid Spot Hybridization (NASH) to detect geographical isolates. Isolates displaying different symptoms were collected on 'Alpon' (AAB) in Bihar, 'Amrithpani' (AAB) in Kovvur, 'Monthan' (ABB) in Bhuvaneshwar, 'Grande naine' (AAA) in Maharastra, 'Namaran' (AAB) in Kolli hills, *Musa ornata* in Tamil Nadu and 'Virupakshi' (AAB) in Pulany hills. The DNA was isolated[1] from mid-rib samples. PCR was performed at an annealing temperature of 53ºC in eppendorf master cycler-gradient. NASH was performed according to the protocol of the manufacturer (Roche Diagnostics).

DAC-ELISA did not detect the viral isolates. The PCR method detected all the isolates except for one. A dig-oxigenin labeled non-radioactive probe was developed from a clone containing BBTV componant 3. The DNA was labeled using the random primer method. The isolated DNA was immobilized onto a nylon membrane (positively charged) using blotting apparatus. The membranes were baked at 80ºC for 2hours and stored at room temperature. Membranes were pre-hybridized and hybridized and washed. After blocking and antiserum reactions the membranes were exposed to luminescent film and developed. The infected samples were positive and the healthy one were negative. The NASH technique is more sensitive than DAC-ELISA and PCR and its use to detect the virus in latent form is also discussed.

1. Gawel N.J. and R.L. Jarret. 1991. A modified CTAB DNA extraction procedure for Musa and Ipomea. Plant Mol. Reporter, 9:262-266.

P131 - Preliminary survey of the banana leaf roller, *Erionota thrax*

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The larvae of the banana leaf roller, *Erionota thrax* (Lepidoptera: Hersperiidae) have been reported as minor to important pest in Southeast Asia and Papua New Guinea.[1,2] In severe infestations, the leaf surface area of the banana plant can be drastically reduced. Several parasitoids with the potential to control this pest have been reported.[3,4] In Malaysia and Indonesia, outbreaks are common after a drought and in wind protected areas.[1]

A survey was carried out in a mixed commercial banana plantation throughout January 2004. Leaf rolls were collected weekly from 30 plants in 10 blocks. The parasitoid adults were captured after they emerged from the parasitized larvae and pupae. One hymenopteran parasitoid parasitizes mature larval instars while another one is specific to the pupae. No egg parasitoids were isolated. The percentage of larvae and pupae parasitized was 20.7% and 19.2%, respectively. The level of parasitization was higher in the organically managed blocks. The results suggest that the parasitoids contribute to keeping the leaf roller population in check and could be used in an integrated pest management strategy.

1. Kalshoven L.G.E. 1981. Pests of Crops of Indonesia. P.T. Ichtiar Baru-Nun Hoeve, Jakarta, 701pp.

2. Sands D.P.A, M.C. Sands and M. Aruna. 1988. A survey of the abundance, distribution, and natural enemies of *Erionota thrax* in Papua New Guinea, June – July 1998. CSIRO Division of Entomology, Australia.

3. Hasyim A. N. Hasan, H. Syafril and K. Nakamura. 1994. Parasitoids of the banana skipper (*Erionota thrax*) (L) in Sumatera Barat, Indonesia, with notes on their life history, distribution, and abundance. Tropics 3(2):131-142.

4. Waterhouse D.F. 1993. The major arthropod pests and weeds of agriculture in S.E. Asia. Australian Centre for International Agricultural Research, Canberra 141pp.

P132 - Use of DNA polymorphism to differentiate non-pathogenic Fusarium isolates

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Endophytic Fusarium isolates recovered from roots of wild banana plants are difficult to characterize beyond the species level based on their morphological characteristics. Differentiation of *formae speciales* and races often requires laborious pathogenicity tests. In this study, the sampled isolates had to be distinguished from the pathogenic *Fusarium oxysporum* f. sp. *cubense* race 1 and 4 (FocR1 and FocR4) to facilitate the selection of a 'nonpathogenic' isolate with antagonistic properties as a potential biocontrol agent against FocR4. To achieve this, a randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique was utilized. The DNA of each fungal isolate was amplified using oligonucleotide primers of OPC 11 (5'-AAAGCTGCGG-3'), OPC 14 (5'-TGCGTGCTTG-3') and OPC 15 (5'-GACGGATCAG-3') to generate fungal genomic fingerprints. FocR1 and FocR4 isolates were also fingerprinted as reference isolates.

Four isolates (PR1, PR2, PR3 and PR4) were more closely related to the pathogenic FocR1 and FocR4 than to the other 67 isolates that were distributed over eight clusters. However, they were not in the same sub-cluster as FocR1 and FocR4. Pathogenicity tests conducted with the susceptible host 'Pisang berangan' revealed the nonpathogenic status of isolates PR1, PR2, PR3 and PR4. Results from this study also suggested that FocR1 and FocR4 were not widely present in the roots of wild bananas, as they were not recovered from the Fusarium population. Nonpathogenic forms were the more dominant endophytes.

P133 - Use of GUS and GFP fusion system to monitor the spatial distribution of *Fusarium oxysporum* in banana roots

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This study was conducted to monitor, using a detectable marker, the spatial distribution of nonpathogenic isolates of *Fusarium oxysporum* (Fo4) that had been shown to induce some form of resistance against *Fusarium oxysporum* f. sp. *cubense* (Foc). Fo4 was transformed with a GUS gene fusion system (*Escherichia coli* β-D-glucuronidase gene) and green fluorescent proteins (GFP). These genes were inserted into the Fo4 genome through *Agrobacterium tumefaciens*-mediated transformation method with hygromycin B resistance gene as marker. GUS was detected by 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc). The stability of the transformed Fo4 in culture over a period of five conidiation cycles was assessed based on the cultural and morphological description, antagonistic activity towards Foc and DNA polymorphism.

No significant difference ($P>0.05$) in the cultural and morphological characteristics of GUS-transformed Fo4 and Fo4 were observed. Both cultures produced white to brown color pigmentation on potato dextrose agar, presence of oval-ellipsoid microconidia (2 x 2–6μ) produced on simple sickle-shape conidiophores and fusoid three to five septate macroconidia (3-4 x 8-12μ). Globose chlyamydospores were produced in older cultures. The antagonistic activity was maintained at values within the range of 40.9 –44.3% based on the percentage inhibition of radial growth (PIRG) *in vitro*.

Spore suspension of the GUS-transformed Fo4 was incorporated into the growth medium to study the distribution of the antagonist in the medium and its ability to colonize banana roots. Re-isolation of the introduced antagonist was determined by serial dilution plating on peptone PCNB agar amended with X-Gluc, whereby the colonies of the GUS-transformed Fo4 were distinguished by their blue color. The frequency of re-isolation at day 2 was 17.2 cfu/g substrate, which showed a reduction of 33.0% from day 1, however the cfu counts remained stable with a gradual increase over a period of 8 days. However, the cfu counts increased with time on the roots of banana suggesting that they were moving from the growing medium to the roots, colonizing the germinating roots, possibly in response to the root exudates. GUS-transformed Fo4 could be detected inside root tissues 2 days after inoculation, however the cfu counts, ($3.6x10^2$/g air-dried roots) were much lower than the values ($7.5 x10^4$/g air-dried roots) detected on the root surface. Similar observations were noted in the distribution pattern of Fo4 in the growing medium and in and on banana roots. This study suggests the possibility of using GUS and GFP fusion technique as a detectable reporter gene to develop a direct system for monitoring the spatial distribution of potential antagonist in and on banana roots. Further studies are currently being undertaken to optimize this approach and to evaluate its potential in banana seedlings challenged with FocR4.

P134 - Morphological characteristics of *Phyllostictina* on banana leaves affected by freckle disease in West Malaysia

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Phyllostictina was examined on banana leaves that had been infected by freckle disease in West Malaysia. Histological examinations of leaf tissue cleared with lactophenol-ethanol revealed immature and mature pycnidia between the cuticle and palisade mesophyll layer. Samples with mature pycnidia were washed under running tap water and cut into 5 cm^2 pieces, placed on moistened filter paper in Petri dishes and incubated at 24±2ºC. Cirri containing conidia exuded from pycnidia after 2 to 4 hours. The dimensions of 30 pycnidia and conidia were determined and compared with data reported previously in literature. Conidia were single celled, hyaline, surrounded by a gelatinous envelope, and varied in shape from obovoidal, ellipsoidal to short cylindrical. Each conidium bore or did not bear a hyaline apical appendage and was readily disseminated in water. Morphological features of the pycnidia and conidia that are useful for identifying the fungus will be discussed.

P135 - Volatiles released by the leaf sheaths of 'Pisang awak'

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Modifying insect behaviour by using non-toxic semiochemicals is recognized as a promising alternative to conventional approaches. Volatiles released by 'Pisang awak' were collected on Tenax adsorbent using an air entrainment technique. The volatiles were eluted with organic solvent. A gas chromatography-mass spectral analysis indicated a host of volatiles. Aspidospermidin-17-0l, 1-acetyl-19, 21-ep, 1-pentatriacontanol, cholestan-3-ol, and 2-methylene (3, beta, 5) were the major components identified in the volatile profiles. The concentrated volatiles were tested against banana stem weevils under laboratory conditions.

P136 - Comparison of *Mycosphaerella fijiensis* isolates from organic and conventional plantations

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The major banana disease is black leaf streak disease, which is caused by *Mycosphaerella fijiensis* Morelet and damages the leaves. In Ecuador, the disease was firstly reported in 1987 in Cavendish plantations. From there, the disease spread rapidly throughout the country and affects 180 000 ha. Usually, disease control is based on alternating systemic and contact fungicides. About ten years ago, 10% of the banana growing areas were converted to organic management. In this system, the disease is controlled by the application of biological products and organic solutions, soil management, stimulation of biological activity, efficient drainage, balanced nutrition, removal of infected leaves, etc. The objective of this study is to compare the physiological and pathogenic behaviour of fungi from organically managed plantations with the one of fungi from conventional plantations. Preliminary results are based on *in vitro* and *in vivo* assays. The populations from organic plantations showed a higher conidia production and aggressiveness under controlled and field conditions than the fungi from conventional populations. To evaluate possible genetic changes in these fungal populations, molecular results will be shown.

P137 – Potential markers for Fusarium wilt

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Field evaluation of Fusarium wilt has not been effective as the infection period is slow and other variables, such as the distribution of inoculum is difficult to control. A study was conducted to develop a semi-qualitative bioassay for early and rapid susceptibility tests against Fusarium wilt disease in banana plantlets. *Fusarium oxysporum* f.sp. *cubense* VCG 01217 was induced to sporulate and germinate and the effect of inoculum density on the infection of 'Rastali' (AAB) plantlets was determined. The concentration of hydrogen peroxide and other enzymes such as phenylalanine ammonia lyase, chitinase, glucanase, peroxidase and polyphenol oxidase in infected roots were determined and the levels related to tolerance or susceptibility to Fusarium wilt. The use of these biochemical parameters has the potential to predict levels of tolerance or susceptibility to Fusarium wilt.

P138 - Preliminary characterization of banana mild mosaic virus isolates from Colombia, the Philippines and Burundi

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A study was conducted to characterize the protein coat of banana mild mosaic virus (BanMMV) isolates infecting *Musa* spp. in Colombia, the Philippines and Burundi, for diagnostic purposes. Two BanMMV isolates from Colombia, one from the Philippines and one from Burundi were detected by immuno-capture-reverse-transcription-polymerase chain reaction. The amplified cDNA fragments were treated with *Eco* RI and sequenced. Amino acid (aa) sequence analyses of the protein coat of these isolates, revealed a highly variable region (aa 8-13) when compared to the type BanMMV isolate (AF314662). The protein coat of one BanMMV isolate from Colombia, and one from the Philippines, contained a restriction enzyme site at nucleotide position 334, which was not present in other BanMMV isolates. Phylogenetic analyses of the alignment of the protein coat aa sequences, revealed that the BanMMV isolates from Colombia, the Philippines and Burundi clustered with the type BanMMV isolate, and were most closely related to the monophyletic group of Cherry green ring mottle virus and Cherry necrotic mottle virus, both members of the *Foveavirus* genus.

P139 - Preliminary molecular characterization of nematode isolates from Malaysia

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Nematode species are usually identified on the basis of a combination of morphological characters. However, identification is made difficult by extensive morphological variation within species and molecular-based identification methods are needed. Sequence comparison of nuclear ribosomal DNA (rDNA) has been effective as a means of clarifying phylogenetic relationships. In this study, a set of universal rDNA primers were used to assess the diversity of nematode isolates collected in untreated soils throughout peninsular Malaysia and belonging to various genera. The primers targeted the 18S and 5.8S genes in the tandem rDNA repeat, amplifying the first internal transcribed spacer (ITS 1) located between the genes. Using PCR, we amplified the ITS 1 region.

P140 - Potentially suppressive soils from northern Malaysia

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Fusarium wilt disease of banana is a serious problem in Malaysia. Therefore, intensive efforts are made to develop a management strategy to control the disease. Studies on several soil-borne plant pathogens have shown that other microorganisms resident in the soil can have an influence on the incidence and severity of disease. The occurrence of soils that suppress the development of *Fusarium oxysporum* f.sp. *cubense* was studied. A survey conducted in northern Malaysia pointed to potentially suppressive soils in certain areas. All the farms visited were infected with Fusarium wilt but no infestation was observed in Gunung Beseri and Semadung. The degree of severity was highly influenced by plant age, soil type and cultivar. The objective of this study was to associate soil type with the occurrence and severity of the disease.

Disease was severe in sandy soils compared with clay and river alluvium soil. The incidence of Fusarium wilt was nil in soils with a pH close to neutral. The chemical properties of the soils suggest that the potentially suppressive soils have higher levels of calcium, manganese and iron.

About 33 strains of bacteria and fungi were isolated from various depths of purportedly suppressive soils. Isolates were screened using *in vitro* antibiosis technique to the pathogen on SGA medium. The results suggest that several isolates from Beseri Jaya and Semadung were responsible for the suppression of the Fusarium wilt pathogen. Further evaluation was made by removing the suppressive properties of the soils by sterilization. The results will be discussed.

Session 3

Sustaining natural resources base in *Musa* cropping systems

Oral presentations

Keynote lecture: Banana specific microbial communities and development of suppressive plants through biological enhancement technologies

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Research has demonstrated that plants lose up to 37% of their assimilates to the soil. These highly nutritive exudates move between the cells in the endorhiza onto the rhizoplane before leakage moves them into the rhizosphere. The production of these nutrients utilizes significant amounts of plant energy, which are ultimately lost to plant growth. I take it for granted that this enormous amount of energy loss occurs in the natural production cycle of a banana plant. Plants have evolved over millions of years and have adapted to present day environments. Plants therefore do not expend this amount of energy without a reason. Why does a plant, in this case banana, exert large amounts of energy to produce nutrients that land unused in the soil?

In my opinion, bananas do not expend energy willingly. In fact I would be willing to venture that under "healthy conditions" nutrient loss is minimal. I believe there are two reasons for the existence of massive leakage into the rhizosphere: (1) roots are damaged by pests and diseases and can not utilize the nutrients produced in the shoot and (2) the plant has evolved a health support system made up of rhizosphere specific microbial communities (RSMC) that live in symbiotic and/or mutualistic associations on these nutrients. These RSMC are not enhanced by standard banana production systems even though they are important for root health and growth. Research has shown that the interactions between banana and mutualistic endophytic fungi (MEF) and/or arbuscular mycorrhizal fungi (AMF) are important for root health and growth. This interaction has been studied in detail and these forms of microbial communities have evolved concomitantly with the plant over evolutionary time. We have shown that specific fungi and probably even bacteria that have plant health promoting abilities are important for root health. When an ecological state is reached in which RSMC are well established and are functioning properly we believe this leads to a disease or pest suppressive agro-ecosystem.

Questions that will be discussed in this talk include: 1) do rhizosphere specific microbial communities exist in banana that affect pest and disease suppressiveness, 2) can this antagonistic potential be measured or detected in the field, 3) can these organisms be isolated, 4) is it possible to produce and re-inoculate these RSMC onto banana plant tissue to produce pest and disease suppressive plantations and 5) is the process of biologically enhancing banana planting material cost effective?

Keynote lecture: Actual and potential soil quality constraints in East African highland banana systems and their relation with other yield loss factors

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In East Africa, highland banana yields (5-30 t $ha^{-1}yr^{-1}$) are low when compared to potential yields (50-70 t $ha^{-1}yr^{-1}$). This is commonly attributed to pests, diseases, soil fertility decline, and occasional droughts. Although there is virtually no quantitative data to prove that soil fertility declined, foliar samples and fertilizer trials have shown that K, N and Mg deficiencies are common, but little information is available on micronutrient deficiencies.

With the urban population steadily growing, banana products and their nutrients are increasingly exported from the farms to the urban centers. Contrary to most commercial banana farmers elsewhere in the world, East African banana farmers seldom use mineral fertilizers to replenish soil nutrients. Instead, they only rely on manure and crop residues, causing further soil fertility decline of grassland and annual cropped fields. In addition, the availability of organic inputs is decreasing due to increasing land pressure. In the long term, sustaining soil fertility without some use of mineral fertilizers seems unlikely. High fertilizer prices, poor availability of credit and a lack of knowledge on how and what fertilizer are best used, currently hamper adoption of mineral fertilizers. There are also no recommendations on the minimum mulch quality and quantity needed to address soil fertility and drought problems in highland banana systems.

There is evidence showing that pest and disease pressure are closely related to soil fertility status and plant nutrient uptake, but the functional relationships between these factors are still poorly understood. Field trials have shown that soil fertility interventions in existing plantations are not economic when pest pressure is high. When developing improved crop management options, we need to look at the farm level and address both pest and disease and soil problems in an integrated way in order to enable successful adoption and sustainable production.

Response of tissue cultured banana cv. Robusta (AAA) to varying levels of N, P and K

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In recent times, there has been interest in the use of *in vitro* produced planting material. Reports point to the superior performance of tissue culture plantlets, compared to suckers[1]. Banana requires high levels of nutrients for proper growth and production. It is estimated that a crop of fifty tonnes in one hectare removes 320 kg of N, 32 kg of P_2O_5 and 925 kg of K_2O every year[2]. Very little work has been done on the growth dynamics, culture and nutrition of banana plants obtained through tissue culture. Hence, a field trial was conducted during 2000-2002 in College Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-3 to standardize fertilizer requirement for tissue culture plants of 'Robusta' (AAA) both in the mother plant and ratoon crops. There were six treatments replicated four times in a randomized block design. The treatments consisted of three levels of application: 100%, 150% and 200% of recommended NPK (110:35:330 g per plant) applied in three and four splits (3, 5 and 7 months after planting and 2, 4, 6 and 8 months after planting).

Application of 165:52.5:495 g of NPK per plant in four splits (150% of the recommended dose) resulted in vigorous plant growth with higher girth, more leaves and greater leaf area. Days taken from planting to shooting in plant crop (ratooning to shooting in case the of ratoon crop), shooting to harvest and total duration were also significantly reduced and bunch weight increased.

1. Robinson J.C. 1990. A field comparison of conventional suckers with *in vitro* derived banana planting material in the first crop cycle. Acta Hort. 275:181-186.

2. Lahav E. and D.W. Turner. 1983. Banana nutrition. Int. Potash Inst. Bull. 7:33.

Review of on-station and on-farm research on the root system in Nigeria and Uganda

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Extensive research on the *Musa* spp. root system of field-established plants was carried out on-station at the IITA Onne High rainfall station, Nigeria and at both the Kawanda Agricultural Research Institute and the Makerere University Agricultural Research Institute Kabanyolo in Uganda. Additional on-farm research on root development was carried out in benchmark sites in Masaka and Bushenyi in southwestern Uganda. A total of 46 genotypes belonging to 12 *Musa* spp. genomic groups and 3 ploidy levels were assessed in field trials in Nigeria. Both sucker and *in vitro*-derived plants were assessed, while special attention was paid on the AAB plantain genotypes. Research trials carried out in Uganda assessed 31 genotypes belonging to 9 *Musa* spp. genomic groups and 3 ploidy levels. The East African highland bananas (AAA) got special attention.

Results at both locations were in agreement. Bunch weight, root, corm and aerial plant growth traits were significantly interrelated. In addition, significant genotypic effects were observed on shoot, corm and root traits. However, there was no genotypic effect on the shoot-root ratio indicating that altering the plants balance of dry matter distribution through breeding may be cumbersome. Significant biophysical effects on root system development were observed, while age of the plantation enhanced the high mat phenomenon (i.e. part of the corm grows out of the soil) and reduced the mats' sucker (i.e. lateral shoot) vigour and the root system size. Two simple and non-destructive root system assessment methodologies were obtained in order to help breeders and nematologists in their assessment of *Musa* spp. landraces and hybrids.

Effect of clean planting material on agronomic parameters and nematode damage

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Nematodes are usually transmitted to new fields through infested planting material. Methods for cleaning planting material exist but they are rarely applied because they are costly and labour or resource-intensive. An experiment was conducted in southern Rwanda with the local East African highland banana 'Mbwaziruma' (AAA) to compare the effect of cleaning methods on damage due to *Pratylenchus goodeyi* and banana productivity.

The three treatments were: paring of suckers before planting; paring and solar drying for three days at 25-30^{o}C; and paring and hot water treatment for 20 min at 55 ^{o}C. The farmers' way of planting rooted suckers was the control. Plant height and girth at 1 m from the ground at flowering, number of functional leaves at flowering and harvest, time to flowering and fruit filling, bunch weight of mother plant crop and first ratoon crop, number of dead roots, % of root necrosis and densities of *P. goodeyi* were recorded regularly.

Time to flowering and fruit filling were similar in all treatments. Bunch weight increased by 17% in sun-dried, 9.3% in hot water treated plants and 6.9% in pared plants, but there was no significant difference between bunch weights in the first ratoon crop (Table 1). Root necrosis and densities of *P. goodeyi* tended to be highest in the control plots and lowest in the hot water treated plants (Table 2). Paring and solar drying improved root health up to 21 months after planting and hot water treatment up to 25 MAP. Field studies in Uganda with R*adopholus similis* and *Helicotylenchus milticinctus* had reported periods of 30 to 36 months[1].

By the end of first ratoon, root health in all treatments was similar to the one in control plots. Densities of *P.goodeyi* were not correlated with any agronomic parameters but root necrosis was negatively correlated with plant girth ($r=-0.206$, $p<0.001$), number of functional leaves at flowering ($r=-0.179$, $p<0.01$), harvest ($r=-0.16$, $p<0.01$) and bunch weight ($r=-0.268$, $p<0.001$) in the mother plant crop. The % of dead roots was positively correlated with time to flowering ($r=0.335$, $p<0.001$), fruit filling ($r=0.385$, $p<0.001$) and plant height ($r=0.318$, $p<0.001$) and negatively correlated with bunch weight ($r=-0.116$, $p<0.05$). Cleaning methods have short-term impact on banana productivity and their application should be combined with other treatments to achieve a long-term impact.

1. Speijer P.R., C.S. Gold, E.B. Karamura, B. Goossens, A. Elsen and D. De Waele. 2000. Rate of nematode infestation of cleaning planting material (*Musa* spp. AAA) in Uganda. Pp. 461-467 *in* Proceedings of the first international conference on banana and plantain for Africa. (K. Craenen, R. Ortiz, E.B. Karamura and D.R. Vuylsteke, eds). Kampala, Uganda, 14-18 October, 1996. NARO-IITA-ISHS, Acta Horticulturae 540.

Table 1. Effect of cleaning method on agronomic parameters (Means followed by the same letter are not significantly different at $p<0.05$ according to the Least significant difference test).

	Girth (cm)		Height (cm)		Number of leaves at flowering		Number of leaves at harvest		Bunch weight (kg)	
	MP	R1	MP	R1	MP	R1	MP	R1	MP	R1
Pairing	71.7 a	60.2 a	289.9 a	340.5 a	9.8 a	8.1 a	3.2 a	3.8 a	12.8 a	13.0 a
Pairing + solar drying	71.5 a	59.6 a	288.6 a	332.8 a	10.2 ab	8.1 a	3.2 a	4.2 a	13.7 ab	11.9 a
Pairing + hot water	70.7 a	60.4 a	281.0 a	333.3 a	10.7 b	8.1 a	3.0 a	4.1 a	15.0 c	13.5 a
Control	69.4 a	59.5 a	284.3 a	333.3 a	9.8 a	8.3 a	2.9 a	4.1 a	14.0 bc	12.9 a

MP=mother plant
R1=first ratoon crop.

Table 2. Effect of cleaning method on root health (Means followed by the same letter are not significantly different at p<0.05 according to the Least significant difference test).

	% root necrosis		% dead roots		Number of *Pratylenchus goodeyi* per 100 g fresh roots	
	5 MAP	32 MAP	10 MAP	32 MAP	18 MAP	29 MAP
Pairing	3.8 a	14.5 a	9.9 a	53.4 a	59.7 ab	57.5 a
Pairing + solar drying	5.8 a	25.8 a	8.2 a	51.4 a	164.6 b	28.0 a
Pairing + hot water	2.6 a	10.2 a	9.8 a	54.1 a	15.7 a	22.0 a
Control	5.1 a	18.7 a	18.9 b	45.7 a	197.2 b	37.8 a

MAP=months after planting.

Is foliar spray better than soil application of micronutrients in banana under high soil pH condition?

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Micronutrients were applied to banana plants through either soil or foliar application. In soils where pH is greater than 8.5, the availability of micronutrients is low even if the concentrations are naturally high or increased through fertilization. Soil and foliar applications of micronutrients in high pH soil have never been compared using bananas. The experiment was conducted with 'Karpuravalli' using a 3^3 factorial design with three levels (0:control, 1:soil application of 5 g per plant of $FeSO_4$, $ZnSO_4$ or Borax, and 2:foliar application of 0.5% $FeSO_4$, 0.5% $ZnSO_4$ or 2ppm boric acid). A common N, P_2O_5 and K_2O dose of 200, 50 and 400 g/plant, respectively, was given to all the plants. The treatments were replicated thrice with eight plants per replicate. Soil applications were made 3 months after planting, whereas foliar applications were made 3, 5 and 7 months after planting. The highest bunch weight (16.3 kg), which is 56% more than that of the control, was observed with a soil application of Fe and foliar applications of Zn and B recorded (Table 1). The effect of soil and foliar applications of micronutrients on macro and micronutrient concentrations in leaves and on fruit quality will be discussed.

Table 1. Effect of soil and foliar applications of micronutrients on agronomic parameters of 'Karpuravalli'.

Levels of Fe, Zn and B	Pseudostem height (cm)	Pseudostem girth (cm)	Number of leaves at harvest	Leaf area at flowering (cm^2)	Number of fingers per bunch	Number of hands per bunch	Bunch weight (kg)
0,0,0	182	79	20	10.8	146	10	10.4
0,0,1	242	71	19	11.3	102	11	10.3
0,0,2	218	76	30	10.7	145	11	10.4
0,1,0	227	72	31	11.2	163	9	10.1
0,1,1	258	90	33	12.2	124	10	10.3
0,1,2	277	91	33	12.4	195	11	10.9
0,2,0	272	90	33	12.0	242	14	12.8
0,2,1	287	93	33	13.1	237	15	14.6
0,2,2	298	99	36	12.9	184	13	12.4
1,0,0	302	97	32	13.0	193	12	11.6
1,0,1	320	96	33	12.8	220	13	10.3
1,0,2	321	99	32	12.4	168	11	11.2
1,1,0	318	99	32	11.7	205	12	11.3
1,1,1	320	99	34	13.0	209	13	13.9
1,1,2	319	98	33	13.2	195	12	15.0
1,2,0	321	99	35	13.6	211	13	14.9
1,2,1	313	98	32	14.0	210	13	15.6
1,2,2	328	101	35	14.6	218	14	16.3
2,0,0	288	96	32	12.9	232	14	13.6
2,0,1	244	92	31	12.8	216	13	12.8
2,0,2	296	97	29	13.6	192	13	13.7
2,1,0	315	100	31	12.7	203	12	14.8
2,1,1	298	99	33	12.0	179	13	13.4
2,1,2	282	99	31	12.6	185	13	12.8
2,2,0	309	98	35	12.0	188	12	12.2
2,2,1	307	100	34	12.7	202	13	12.1
2,2,2	301	98	34	14.2	210	14	14.6
CD	44.1	12.7	2.4	2.91	56.9	2.4	2.03

0:control, 1:soil application, 2:foliar application

CD: Critical difference at p=0.05.

Response of fertigation on certain cultivars of banana under different planting densities

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Fertigation is a technique that combines fertilizer application with any micro-irrigation system, especially through drip irrigation. The success of fertigation depends upon timing the quantity of fertigation with the stage of the crop. Studies at the Horticultural College and Research Institute, Coimbatore, have revealed that the scheduling of fertilizers at weekly intervals is adequate and NPK are to be distributed at various stages as follows:

Weeks 9 to 18	30% N	100% P	20% K
Weeks 19 to 30	50% N	-	40% K
Weeks 31 to 42	20% N	-	32% K
Weeks 42 to 45	-	-	8% K

Based on the above schedule, the effect of fertigation was tested with 'Robusta' with 50% (F1), 75% (F2) and 100% (F3) of recommended doses of N,P and K fertilizers under the following planting densities: D1 (one sucker/pit, 1.8 m x 1.8m, 3086plants/ha); D2 (2 suckers/pit, 1.8 m x 1.8 m, 6172 plants/ha); D3 (3 suckers/hill, 1.8 m x 3.6 m, 4500 plants/ha) and D4 (paired row system, 1.2 m x 1.2 m x 2 m, 5200 plants/ha). The largest bunches were obtained with 100% fertigation. Increasing the density reduced bunch weight. The highest yield, 136 t/ha, was registered with D2F3. However, the highest yield with a good bunch was recorded with D3F3. With 'Red banana' (AAA) the largest bunches (22.5 kg) were recorded under D1F1 but the highest yield (98 t/ha) under D2F1. The effect of fertigation on morphological traits, crop duration besides on certain physiological parameters on these cultivar of banana will be discussed.

Modified high density planting and fertigation

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The conventional practices followed in India with respect to planting densities, fertilizer application, mat removal is highly labour and input intensive. The cost of production under the present system of the cultivation has to be drastically reduced in order to have a competitive edge in the domestic as well as international market. The present study was done to increase the production and productivity of 'Robusta', to increase the efficacy of inputs through fertigation and to study the impact of modified high density planting on the incidence of pest and diseases. Four densities were tested: conventional planting at a spacing of 1.8 m x 1.8 m (3086 plants/ha), two suckers/hill at spacing of 1.8 m x 3.6 m (3086 plants/ha), three suckers/hill at 1.8 m x 3.6 m (4500 plants/ha) and paired row planting at 1.2 m x 1.2 m x 2 m (5200 plants/ha) with three fertigation levels of N and K (100%, 75% and 50% of recommended dose, ie. 200 and 300g per plant, respectively).

Planting 3 suckers/hill with 75% N and K fertigation (150:30:225g NPK/plant annually) and the paired row system with 100% N and K fertigation (200g N, 30g P and 300g K) recorded the better growth parameters. Flowering was 2 weeks earlier with the paired row system. Bunch weight was not influenced by the modification of the density. Significant variations however, were observed among fertigation levels and 100% N and K fertigation recorded the highest bunch weight (18 kg). Covering of bunches with white polythene sleeves with 6% ventilation resulted in uniform and attractive light green colour in fruits compared to uncovered bunches. Blemishes over the fruits were also less in covered bunches. *Mycosphaerella* leaf spot incidence was lowest with 3 suckers/hill and highest in conventional planting system. Nematode population was however, high in 3 suckers/hill followed by 2 suckers/hill and the lowest in paired row system. Both density and fertigation levels did not influence the green life and yellow life of fruits. Physiological loss in weight of fruits was influenced by the level of fertigation. The paired row system (5200 plants/ha) with 100% N and K fertigation (200:30:300g NPK/plant annually) was more economical with high benefit- cost ratio of 2.02 followed by 3 suckers/hill with 75% N and K fertigation (1.74).

Effect of intercropping on yield parameters and soil fertility

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The effect of intercropping on the yield and fruit quality of 'Giant governor' (AAA) was assessed at the Horticultural Research Station in Mohanpur, India, between 1997 and 2000. Banana was intercropped with amaranthus, cowpea and sweet potato; elephant foot yam, pea and ricebean; ginger and lathyrus; turmeric and cowpea; bhindi, chilli and marigold; pumpkin, cowpea and marigold; greengram, ricebean and tomato; and brinjal and gladiolus. Banana intercropped with turmeric and cowpea produced the largest bunches (18 kg) and highest yields (48.44 t/ha). The highest finger weight (140 g), total soluble solids (brix of 20), total sugar content (16%) and highest benefit/cost ratio (1.4) were also observed with this cropping system. The soil nitrogen status was appreciably higher with cowpea while the available phosphorus and potassium content in the soil increased with cowpea and ricebean.

Effect of potassium nutrition on growth and nutrient uptake of banana plants

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Plants differ markedly in their need and ability to absorb and translocate a specific nutrient in comparison to other nutrients. Bananas need fairly large amount of potassium for their growth and productivity. Despite the recommended fertilizer practices, growth and yield of banana are declining. The reason might be its large K uptake capacity to replace the K removed in the harvested fruits and other plant parts. Large quantities of K leaching owing to low exchange capacity of tropical soils coupled with rapid oxidation of soil organic matter and the lower efficiency of tropical soils for applied fertilizer (estimated at around 40%[1] for K) are among the other factors responsible for the reduction in banana growth leading to decreased yield and profit of the farmer. This paper discusses the effect on nutrient uptake (N, P, K, Ca and Mg) and growth of increasing K application in 'Berangan'.

We studied the performance of varying K rates, low (50 g K_2O/plant), optimal (300 g K_2O/plant), and high (600 g K_2O/plant), on banana. The plant responses were examined in terms of leaf area, pseudostem girth, stem height and leaf nutrient concentration. Tissue-cultured banana plants were used as planting material grown in 76x76 cm polythene bags filled with 100 kg of Serdang series soil (3:2:1 ratio) for five months with different concentration of K, the other nutrients were applied equally to all plants[2]. Data on growth parameters were recorded fortnightly and leaf nutrient analysis of third fully expanded leaf from the top was analysed for N and P using auto analyser and K, Ca and Mg by Atomic Absorption Spectrophotometer method.

Increasing the rate of K (600 g K_2O/plant) significantly produced 40, 33, and 26% more leaf area, pseudostem girth, and plant height, respectively, compared to plants grown in low K treatments. Plants supplied with optimum K rate (300 g K_2O/plant) also yielded 29, 24 and 21%, more leaf area, stem girth and plant height, respectively, compared to K deficient plants. A general retardation of pseudostem height, circumference and smaller leaves with shorter longevity have also been reported[3]. Plants with higher K status resulted in 16, 11 and 6% increased leaf area, stem girth and plant height, respectively, indicating some positive effects of increased K nutrition on overall growth of banana.

K deficiency reduced leaf K concentration below the critical level estimated for banana, followed by reduction in N and P concentration[4]. On the other hand, the concentration of Ca and Mg increased predominantly. As in K deficiency other cations seems to partially replace the role of K and the plant starts to absorb large amounts of other cations, usually Ca and Mg. However, in this study the growth reduction caused by K deprivation could not be compensated by higher concentration of Ca and Mg, which was evident from the poor growth performance in K-deficient treatments. High K supply enhanced the leaf nutrient concentration of N compared to K-deficient plants, no effect on P, and reduced the concentration of Ca and Mg. The concentration of Ca and Mg, however, were not below the critical value that can cause deficiency as no deficiency symptoms of Ca and Mg were observed in high K-supplied plants. In plants with optimal level of K, the concentration of N and K was normal but not adequate for vigorous growth and high yield as mentioned in the literature. A depressive effect on leaf area was also observed with rapid senescence and death of the old leaves due to K deficiency in the low K as well as in some plants from optimum K-fertilized plants, indicating the insufficiency of the present rate (300 g K_2O/plant) for banana and especially for 'Berangan'. The need for increasing K application for increased leaf area has been reported[5]. Larger leaves with increased K nutrition in our study indicated the important role of K in leaf expansion. The results revealed that the application of K in excess of the recommended rates could improve the overall growth performance of banana corresponding to an increase in the rate of N, as the N/K ratio is very important for banana.

1. Baligar V.C. and O.L. Bennett. 1985. NPK-Fertilizer Efficiency- a situation analysis for the tropics. Fertilizer research 10:147-164.

3. Lahav E. and D.W. Turner. 1983. Banana Nutrition. Int. Potash Instit. Bull. 7.

2. Lahav E. 1972. Effect of different amounts of potassium on growth of banana. Trop. Agric. (Trinidad) 49(4):321-335.

4. Turner D.W. and B. Barkus. 1983. Long-term nutrient absorption rates and competition between ions in banana in relation to supply of K, Mg and Mn. Fertilizer Research 4:127-134.

5. Yusoff M.A, M.H. Nik, M. Zabeda and T. Zahara. 1998. Trend in foliar nutrient concentrations and contents, and its implications on leaf area index development and yields in banana, cultivar Berangan. Pp.95-105 *in* Proceedings of the first Banana Seminar.

Session 3

Sustaining natural resources base in *Musa* cropping systems

Posters

P56 - Characterization of banana cultivars, production practices and constraints of production for farmers in banana growing areas of Kenya

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Banana is an important crop in Kenya, grown on about 79 000 hectares, more than for any other fruit. Farmers produce an average of 4 t/ha annually. A Rapid Rural Appraisal (RRA) and Participatory Rural Appraisal (PRA) exercises were conducted between 1996 and 1998 in southwest, coastal, central and eastern Kenya, to identify major constraints, rank them and determine suitable interventions with farmers. The sites were Kenyenya, Suneka, Nyamache, Merigi, Kabondo, Nyamira, Kendu Bay, Murang'a (Gakoigo), Kirinyaga (Gathiga), Embu (Gatituri and Kigaa) and Kilifi and Taveta. Most of the production is by small-scale farmers practising intercropping with other crops like maize, beans and groundnuts. Plantations are established with suckers obtained from neighbours and friends, which carries high risks of transmitting pests and diseases and does not meet the high demand for suckers. Banana is both a security crop and an income earner. Diverse banana cultivars are grown, such as 'Ekeganda' (AAA-EA), 'Ngombe' (AAA-EA), 'Kisukari' (AB), 'Gradi' (AAA-EA) and 'Bogobogo' (ABB) in southwest Kenya. In central and eastern provinces, cultivars such as 'Muraru' (AA), 'Uganda green' (AAA-EA), 'Kampala' (Gros Michel, AAA), 'Israel' (AAA) and 'Kisukari' (AB) are predominant while in coastal areas Cavendish cultivars (AAA) dominate the commercial farms.

Constraints cut across the agroecological zones and include pests and diseases, poor orchard management, soil exhaustation because of land pressure, lack of superior cultivars and socio-economic factors such as poor infrastructure. Sigatoka disease is prevalent in the coastal region while Fusarium wilt, nematodes and banana weevils are prevalent in the upper and lower midland agroecological zones and moles are a menace in the upper midlands and lower highland areas (Table 1). To address some of the major constraints, on-farm evaluation trials are conducted and tissue culture plantlets are distributed.

Table 1. Banana cultivar mapping and constraints identification with farmers in central and eastern Kenya.

Site	Gakoigo (Maragwa)	Gathiga (Kirinyaga)	Kigaa (Embu Ruyenjes)	Gatituri (Embu central)
Altitude (m)		1280-1430	1400-1590	1280-1460
Rainfall (mm)	1750-1800	600-950	1000-2000	1000-1250
Soils	Deep red loan volcanic derived	Well drained extremely deep dusky red to darkish brown friable slightly clay	Red humic deep well drained nitosol	Humic nitosols well drained extremely deep, dusky, red friable clay with acid humic top soil
Crops grown (ranked)	Maize, bananas, Irish potatoes, beans, french beans coffee	Maize, beans, bananas millet pigeon peas	Maize, beans, coffee bananas Irish potatoes	Maize, bananas beans, coffee, Irish potatoes
Cash crops (ranked)	Bananas, coffee, beans maize	Pigeon peas, beans, bananas maize millet	Coffee, maize, beans bananas Irish potatoes	Coffee, maize bananas beans, irish potatoes
Food crops (ranked)	Maize Irish potatoes, beans bananas	Maize, beans bananas millet pigeon peas	Maize, beans, coffee bananas irish potatoes	Maize, beans, irish potatoes bananas
% of land to bananas	25%	25%	12.5%	8%
Cultivars (ranked)	Kampala, Israel, Muraru, Mucuru, Gatumia (Dwarf cavendish)	Kampala, Muraru, Giant Cavendish, Kiganda, Israel	Golden beauty, Kampala, Israel, Muraru, Kiganda	Kampala, Muraru, Kiganda ,Israel, Gicagara
Constraints	Weevils, nematodes, Fusarium, poor marketing structure and prices	Weevils, nematodes, Fusarium, ants, land pressure, planting materials	Weevils, nematodes, planting materials, theft, low yield, lack of management skills	Weevils, nematodes, Fusarium, lack of management skills, declining yield

P57 - Effect of different cropping systems on production cycle periods and harvest index

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The effect of intercropping on plant crop cycle and harvest index of 'Giant governor' (AAA) was assessed at the Horticultural Research Station in Mohanpur, India, between 1997 and 2000. Banana was intercropped with amaranthus, cowpea and sweet potato; elephant foot yam, pea and ricebean; ginger and lathyrus; turmeric and cowpea; bhindi, chilli and marigold; pumpkin, cowpea and marigold; greengram, ricebean and tomato; and brinjal and gladiolus. Bunch emergence was earliest (333 days) when banana was grown as monocrop, followed by intercropping with turmeric and cowpea (338 days) and with elephant foot yam, pea and ricebean (340 days). The shortest time from shooting to harvest was with monocrop banana (79 days), four days later when intercropped with amaranthus, cowpea and sweet potato and 10 days later with pumpkin, cowpea and marigold. The planting to harvest interval was also shortest (413 days) in monocrop banana, 13 days later when intercropped with turmeric and cowpea and 23 days later with pumpkin, cowpea and marigold. The monocrop banana had the highest harvest index (0.41) followed by intercropping with turmeric and cowpea (0.38).

P58 - Modified high density planting techniques in banana

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Earlier work[1] with cv. 'Robusta' adopting a spacing of 2.4 mx 1.8 m with one or two suckers per pit revealed that double planting produced relatively smaller bunches but increased yield. Based on this experience, further research[2] on planting 2 or 3 suckers per pit at various spacings showed that 'Nendran' planted at 2 m x 3 m with 3 suckers per pit resulted in the highest yields without affecting bunch weight. In an experiment aimed at reducing fertilizer requirement under a modified high density planting system, indicated that planting three suckers of 'Robusta' per pit at 1.8 m x 3.6 m with 50% of recommended doses increased productivity by 28%[3]. Four suckers per pit on the other hand reduced plant girth and leaf area and extended the crop cycle by 45 days compared to 3 suckers/pit.

In another trial, a normal density system of one sucker per pit spaced at 2 m x 2 m (2500 plants/ha) was compared to a high density planting of 3 suckers/pit spaced at 2 m x 3 m accommodating (4998 plants/ha)[4]. High density planted increased plant height, number of leaves and leaf area but extended crop cycle. Bunch weight was also lower (19.5 kg) compared to normal density (24 kg). However, the overall yield/ha was higher under high density planting and there was no appreciable difference between fruit quality.

Effect of planting density was also assessed in 'Red banana'. D1 (one sucker/pit, 1.8 m x 1.8m, 3086plants/ha); D2 (2 suckers/pit, 1.8 m x 1.8 m, 6172 plants/ha); and D3 (3 suckers/hill, 1.8 m x 2.7 m, 6172 plants/ha)[5]. Bunch weight decreased with increased density. The highest yields were recorded with two suckers/pit.

In an another trial, the performance of 'Robusta' was compared under 4 systems: D1 (one sucker/pit, 1.8 m x 1.8m, 3086plants/ha); D2 (2 suckers/pit, 1.8 m x 1.8 m, 6172 plants/ha); D3 (3 suckers/hill, 1.8 m x 3.6 m, 4500 plants/ha) and D4 (paired row system, 1.2 m x 1.2 m x 2 m, 5200 plants/ha). Bunch weight was higher under D1 and D3. Yield was higher under D2 (129 t/ha) followed by D4[6]

1. Azhakia Manavalan R.S. and R. Balakrishnan. 1976. Double planting in Robusta banana. Madras Agric. J. 63(1):46-49.

2. Elain Apshra S. 1997. Effect of planting density and spacing on growth and yield of banana cv. Nendran (AAB). M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.

3. Nalina L. 1999. Studies on high density planting in banana cv. Robusta (AAA). M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.

4. Mahalakshmi M. 2000. Water and fertigation management studies in banana cv. Robusta (AAA) under normal planting and high density planting systems. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.

5. Suganthi L. 2002.Fertigation management studies in banana cv. Red banana (AAA) under different planting densities M.Sc (Hort). Thesis, Tamil Nadu Agricultural University, Coimbatore.

6. Anonymous. 2003. Integrated new production system for bananas for export and domestic needs.

P59 - Production of plantain cv. 'Orishele' in an annual high-density cropping system in Côte d'Ivoire

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In Côte d'Ivoire, the first cultural cycle of plantain cv. 'Orishele' was conducted at high densities at Abbe research station (50 km from northern Abidjan) on silt clay soil. High densities (2500 and 3333 plants/ha) on one hand and the impact of black Sigatoka (treated plots with fungicides and control plots without fungicide application) on the other hand, were compared according to Fisher's experimental blocks design, with 6 treatments and 4 replications. Results gotten during vegetative growth showed positive correlation between densities and the height of the plantain trees. At 2500 and 3333 plants/ha, the plantain trees reached at the time of flowering respectively 3.61 m and 3.69 m of height against 3.50 m for the control (1666 plants/ha). The effect of high densities on the growth didn't affect in a significant way the interval plantation flowering and the circumference of plantain trees. Moreover, the early canopy closure (4 months after plantation) owing to high densities shades out durably the weeds. At harvest, the bunch weight was negatively correlated to the densities. However, high density improved significantly productivity with 33.1 t/ha for 2500 plants/ha and 39.5 t/ha for 3333 plants/ha against only 27.5 t/ha for the control (1666 plants/ha). Agronomic parameters studied showed significant effect of black leaf streak disease. None of the three tested densities had reduced the foliar damage on 'Orishele'.

P60 - Production of cv. 'Berangan' under several planting arrangements

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'Berangan' is a popular dessert cultivar. It flowers about 9 to 11 months after planting and the average bunch weight is between 12 and 16 kg. A study was conducted to look at the effect on fruit quality of planting arrangements:

Rectangular planting system, 3 m x 2 m (1666 plants/ha)
Hedgerow system, 4 m x 1.5 m (1666 plants/ha)
Two row system, 5 m x 2 m x 2 m (1428 plants/ha)
Trem system, 5 m x 2m x 1 m (1666 plants/ha)

The highest average bunch weight in the mother plant crop was observed in the rectangular planting system, followed by the two row system (Table 1). The hedgerow and trem system produced significantly lower average bunch weights. Overlapping of canopy reduced yield in the hedgerow, trem system and two row planting system. The same trend was observed in the ratoon crop for the average bunch weight. Yield was highest with the rectangular planting system for both the plant crop and the ratoon crop.

Table 1 Average bunch weight and yield of 'Berangan' under various planting systems.

	Planting distance and density (plants/ha)	Average bunch weight (kg)	Yield (Tons/ha)
Mother plant crop	3 m x 2 m (1666)	16.8 a	27.9
	4 m x 1.5 m (1666)	13.8 b	23.0
	5 m x 2 m x 2 m (1428)	15.6 a	22.3
	5 m x 2 m x 1 m (1666)	13.2	22.0
Ratoon crop	3 m x 2 m (1666)	16.2	27.0
	4 m x 1.5 m (1666)	12.5	20.8
	5 m x 2 m x 2 m (1428)	14.9	21.3
	5 m x 2 m x 1 m (1666)	12.8	21.3

P61 - Effect of planting hole size on shoot and root development

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In order to control pests and diseases, researchers recommend planting *in vitro* plantlets or pared suckers. These materials are much smaller than conventional suckers. While the recommended planting hole size for big suckers is 60 cm x 60 cm x 60 cm in East Africa, the smaller planting materials could be established using planting holes of reduced size. This could significantly reduce the labour cost of establishing a new plantation. The aim of this study was to assess aerial, corm and root system development of both *in vitro* and sucker-derived bananas planted in holes of 60 cm in diameter but at a depth of 40 or 60 cm. The study was conducted at the Makerere University Agricultural Research Institute Kabanyolo in Central Uganda. Two East African highland bananas (AAA), 'Entaragaza' and 'Siira', were used in this study. Mats were assessed 24 weeks after planting and at flowering.

Planting hole size did not have a significant effect on the aerial, corm and root traits of the sucker-derived banana mats after 24 weeks. However planting hole size did have a positive and significant effect on corm dry weight and cord root length of *in vitro*-derived banana mats. There was no significant effect of planting hole size on growth traits of both the sucker and *in vitro*-derived banana mats at flowering. There was no significant effect of planting hole size on the root distribution down the soil profile, assessed down to 120 cm. In addition, planting material type did not significantly influence the root distribution pattern down the soil profile. This implies that a smaller planting hole does not impede a vigorous plant growth for both types of planting material. In addition, labour costs for the establishment of a plantation could be reduced by at least 33 %. Further studies are however recommended in order to assess plant growth and stability during subsequent growth cycles.

P62 - Evaluation of ‘Petite naine’ in Mauritius

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Mauritius (20.2°S, 57.3°E) is frequently visited by anticyclones and cyclones. In order to investigate the effect of wind damage on crop performance, a trial was set up at Richelieu Crop Research Centre, under drip irrigation, using ‘Petite naine’. The site is located in a sub-humid zone (mean maximum temperature 30°C, mean minimum temperature 20°C, mean rainfall 600 mm/yr). Twenty-eight tissue-culture plantlets of ‘Petite naine’, spaced at 2.0 cm x 2.5 cm in 7 rows (2000 plants/ha) were grown within a 4 m high artificial Sarlon windbreak (porosity 58%). Their performance was compared with that of 28 plants grown in the open. The plants were on average 46 cm tall, and had a collar girth of 20.6 cm and 6.8 leaves. Observations were made on leaf emission rate, wind damage to the youngest fully expanded leaf tagged at monthly interval and parameters for plant growth, fruit yield and quality.

Due to favourable summer conditions during the first four months, the plants developed vigorously. However, the leaves of the plants that were wind-protected emerged at a faster rate and flowered 3 weeks before the exposed ones, 155 and 176 days, respectively. During the vegetative and flowering stages, two cyclones, with gusts up 90 to 120 km/h respectively, affected the plants. At flowering, the wind-protected plants were significantly taller and wider than the exposed plants but the latter were stouter (circumference/height ratio). The protected plants outperformed the exposed plants with respect to earliness in bunch maturation, number of hands and fingers, bunch weight and fruit size. However, probably because no lower hands were removed, average fruit weight in both treatments was lower (120 to 150 g) than those mentioned in the CIRAD/FLHOR fiche technique. The fruit stalk was heavier in protected plants than in the ones grown in the open (3 kg and 2.0 kg, respectively). The yield difference can be attributed to the prevailing microclimate within the protected structure and to differences in the canopy. Wind-protected plants always had significantly more leaves than the exposed plants. Moreover, leaves in the open senesced and the lamina tips of the small tears dried at a faster rate than protected ones, thus reducing the photosynthetic efficiency of the leaves.

Average fruit weight in each hand was comparable across all hands in wind-protected bunches while those from the open had smaller fruit in the lower hands. Amount of bruised fruit and intensity of bruising (5-20% peel surface or even going down to pulp) was more prevalent in exposed bunches. However, fruits in the open had higher Brix but this was not detected by tasting probably because of comparable Brix/acidity ratio.

Agronomic parameters of ‘Petite naine’ under wind-exposed and protected conditions (*F-test significant at p<0.05).

	Plant height (cm)	Plant girth (cm)	Number of leaves at flowering	Number of leaves at harvest	Numbero f hands	Number of fingers	Bunch weight (kg)	Number of fingers
Wind exposed	159.1	56.7	15.4	7.2	9.8	178.4	21.5	178.4
Protected	181.5*	59.6*	19.2*	9.3*	10.1*	189.4*	28.4*	189.4

P63 - Influence of plant density and nutrition on banana cv. 'Robusta' (AAA) in Karnataka

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Studies on plant density and nutritional requirement of cv. Robusta (AAA) were carried out using densities ranging from 2500 to 5000 plants per hectare. One or two suckers per hill were planted and grown over two production cycles under varying nutrient levels. Planting two suckers/hill and providing them with more nutrients than the recommended dose resulted in increased plant height, pseudostem girth, number of leaves, leaf area and leaf area index. With 270 g of nitrogen and 390g of potash, fruit maturity after shooting was earlier by 27 days, thus reducing the period from planting to harvesting by 60 days. The highest bunch weight ranged from 23.9 to 24.7 kg and 23.3 to 24.3kg when 315g of nitrogen and 390g of potash were applied. The highest yield of 119 t/ha and 123 t/ha was obtained with the application of 315 g of nitrogen and 390 g of potash. The best benefit-cost ratio (1:6) and a yield of 116 t/ha for the motherplant crop and 121 t/ha in the ratoon crop was obtained when 675 kg/ha of nitrogen and 982.5 kg/ha of potash were applied.

P64 - Sustainable banana production through recycling of nutrients of parent pseudostem after bunch harvest

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The experiment was conducted at the Horticultural Research Station, Mondouri in West Bengal between 1999 and 2002 with ‘Giant governor’ to investigate the effect of retaining the motherplant along with the connected and severed sucker(s) in the ratoon crop. A trifactorial completely randomized block design was used with plants spaced 1.8 m apart with four replicates and twelve treatments. The motherplant was either retained untopped after harvest, cut at mid-height or at corm level. One or two connected or severed sucker(s) were retained at shooting. The fresh weight of pseudostem (320 t/ha) increased by nearly 50% when the untopped motherplant was retained, compared with the plant cut at corm level. (213 t/ha). A connected sucker was significantly better to severed sucker(s). Retaining two suckers connected to the motherplant produced a record biomass of 444.5 t/ha and also had beneficial influence on bunch weight (24 kg). Keeping the motherplant intact not only produced larger bunches, it also reduced nutrient requirement by 50%.

P65 - Influence of organic manure on growth and yield of banana

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Banana, an exhaustive user of water and nutrient due to its large rhizosphere, rapid growth and high yielding nature, demands large quantities of nutrients of both organic and inorganic source[1]. Farmers apply essential plant nutrients in large quantities as synthetic inorganic fertilizers since these are cheapest and easily available compared to organic manure. The continuous use of inorganic fertilizers leads to undesirable changes in the soil and environment and ultimately endangering the very sustainability of the farming[2]. In addition, continuous use of chemicals in farming has affected the biosphere, physical condition and the capacity of biological productivity of the soil. In recent years, there is a global awareness among environmentalists, scientists and common people on the environmental degradation of soil – plant relationships leading to organic farming. Under organic farming, use of inorganic fertilizers, synthetic pesticides are discouraged and replaced by the use of only recommended organic manures, minerals and bio-pesticides. Though, there is a general opinion that organic manure or like compounds cannot totally replace the chemical fertilizers, continuous and planned use of organic fertilizers proved to be superior to the inorganic farming practices[3]. This study was conducted to find the influence of different organic manures on growth and yield of 'Rasthali' (AAB) and 'Karpuravalli' (ABB).

The treatments were replicated five times with eight plants per replicate in a randomized block design. The different treatments were: distillery sludge, vermi compost, neem cake and poultry manure: distillery sludge 5 kg/plant and vermicompost 2kg/plant applied at monthly intervals between the 3rd and 7th months after planting, neem cake 2kg/plant, poultry manure 5 kg/plant, distillery sludge 2.5 kg + vermicompost 1 kg/plant, distillery sludge 2.5 kg + neem cake 1 kg/plant, distillery sludge 2.5 kg + poultry manure 2.5 kg/plant, distillery sludge 2.5 kg + vermicompost 1 kg + neem cake 1 kg + poultry manure 2.5 kg/plant applied at 3, 5 and 7 months after planting, and a control (200:50:300 g/plant of NPK at 3, 5 and 7 months after planting. A common dose of 20 g Azospirillum + 10g Phosphobacteria and 20g *Trichoderma viride* were applied to all treatments 3 months after planting. Leaf spot disease severity and nematode soil and root population were recorded 8 months after planting

In both banana cultivars, application of 2.5kg compost + 1kg vermi compost + 1kg neem cake + 2.5kg poultry manure 3, 5 and 7 months after planting was the best treatment with respect to growth and yield parameters. Application of organics increased the soil N, P, K, Ca, Mg, Zn, Fe, Cu, Mn contents and organic carbon while decreased the Na content, E.C and pH of soil in both varieties. Organic fertilized plots recorded higher population of bacteria, fungi and actinomycetes as compared to inorganic fertilization. The incidence of leaf spot disease was less with more YLS-0 and YLS-31 (youngest leaf spotted) in organics compared to inorganic fertilizers application. Soil and root nematodes population and infestation were significantly lower in organically fertilized plants.

Application organic manure improved the soil physical, chemical and microflora populations thereby increasing plant growth and bunch parameters and reducing incidences of nematode and leaf spot diseases. Organic fertilization also reduced the cost of cultivation.

1. Lahav E. 1973. Effects and interactions of manure and fertilizers in a banana plantation. Israel J. Agric. Res. 23, 45-47

2. Sharma S.B. 1988. Effect of different methods and times of manuring on growth and yield of banana. Fertilizer News 32(10):33-35

3. Vargas R, S. Laprade and M. Barquero. 1996. Basic considerations on the composting of organic crop residues. Corbana Revista 21(46):163-77.

P66 - Influence of organic manures on the quality of banana fruits

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This study was undertaken to find the influence of organic manures on fruit quality parameters of 'Rasthali' and 'Karpuravalli' bananas. Different organic manure were applied: distillery sludge 5 kg/plant and vermicompost 2kg/plant applied at monthly intervals between the 3rd and 7th months after planting, neem cake 2kg/plant, poultry manure 5 kg/plant, distillery sludge 2.5 kg + vermicompost 1 kg/plant, distillery sludge 2.5 kg + neem cake 1 kg/plant, distillery sludge 2.5 kg + poultry manure 2.5 kg/plant, distillery sludge 2.5 kg + vermicompost 1 kg + neem cake 1 kg + poultry manure 2.5 kg/plant applied at 3, 5 and 7 months after planting.

Fruit quality parameters were analysed from fruit samples taken from the second hand. In general, the application of organic manure produced better quality fruits. Application of 2.5 kg compost + 1 kg vermicompost + 1 kg neem cake + 2.5 kg poultry manure at 3, 5 and 7 months after planting recorded the maximum T.S.S., acidity, total sugars and starch contents in both cultivars.

In 'Rasthali', maximum T.S.S (29.40^{o}B), acidity (0.59%), sugar acid ratio (49.8), total sugars (25%,) and low starch (3.2%) contents were recorded. For 'Karpuravalli' the maximum values were T.S.S (32.20^{o}B), acidity (0.61%), sugar acid ratio (52.8), total sugars (26.3%) and low starch (3.4%). The lowest quality was recorded in inorganically fertilized fruits.

P67 - Status of organic bananas in India

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With the Green revolution, Indian farmers, who are basically organic farmers, shifted to an agriculture characterized by the use of high yielding varieties, chemical fertilizers and pesticides. Even though, it helped increase productivity, it adversely affected the soil's ecosystem, water and human health. This has become a major concern and consumers started demanding food grown organically.

Organic farming is a method of farming that avoids or largely excludes the use of harmful chemicals such as chemical fertilisers, pesticides and herbicides and use natural resources such as organic matter, minerals and microbes to maintain the ecological balance and to provide stability to production without polluting soil, water and air. Organic farming systems rely on large scale application of animal wastes or farm yard manure, compost, crop rotation, crop residues, green manure, vermicompost, bio-fertilisers, VAM, bio-pesticides and biological control. Organic farming produces are found to be superior in quality and also fetches premium price in international markets.

Organic banana is slowly becoming popular in foreign markets like, European Union, Japan and USA and fetches a premium price. The areas growing organic bananas are slowly expanding and co-operatives are formed for quality production and certification of organic bananas[1]. Only a minority of European super markets carry organic bananas[2] because they are a difficult product and do not have a supply chain.

India, especially the north east, is ideal for growing organic bananas. Since these states are under high rainfall and at higher altitudes, the soils are rich in humus and organic matter. In these areas bananas are grown mostly as a backyard crop and commercially not exploited. By planting high yielding tissue culture plants of superior clones that have export potential and can be grown under organically, forming co-operatives and developing handling and storage facilities and storage, organic bananas could increase productivity.

Another potential areas are the higher altitude growing areas in Karnataka, Kerala and Tamil Nadu. Under this situation this bananas are grown mostly as a shade crop for coffee. Nowadays, due to conversion of coffee estates to organic coffee production, there is a good scope for producing organic bananas under this system.

1. Holderness M., J. Bridge and C.S. Gold. 2000. Pest management in organic systems. Pp. 131-132 *in* Organic banana 2000: Towards an organic banana initiative in the Caribbean (M. Holderness, S. Sharrock, E. Frison and M.Kairo, eds).

2. Haest C. 2000. European market potential for organic produce and supply and quality assurance requirements. Pp. 93-94 *in* Organic banana 2000: Towards an organic banana initiative in the Caribbean (M. Holderness, S. Sharrock, E. Frison and M. Kairo, eds).

P68 - Assessment of the performance of foliar TNF (Trace Nutrient Fertilizer) on plantain production

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The agronomic performances of foliar Trace Nutrient Fertilizer (TNF) have been evaluated on the banana plantain (*Musa* ABB) cv. 'Corne1' under intensive cultivation at CNRA experimental site at Abbè in Côte d'Ivoire. The experiment was conducted through four treatments replicated four times (T0: without fertilization and without TNF; T1: recommended mineral fertilization applied to soil; T2: 0,5 L TNF/ha; T3: 1.0 L TNF/ha). There is no statistical difference between treatments as for the vegetative growth of banana plant (height and circumference). TNF had a positive effect on sucker production. Treatment 3 gave a gain of 32.1% and 16.60% in relation to T0 and T1 respectively. According to the variance analysis applied to the average bunch weight, TNF had no effect on the output of plantain plants. However an increase in weight of 5.13% for T2 was observed by comparison with the recommended mineral fertilization; this one is 8.6% in relation to the control. T3 gave heavier banana fingers that those obtained in the other treatments (T3:262.7 g against T2: 248.5 g; T1:246.8 g and T0:233,8 g). The weak response of plantain production to the TNF application could be due to the high level of the soil fertility of the experimental site (the land was under fallow of *Chromolaena* since more than five years). The effect of soil organic matter would have hindered the effect of treatments. Plantains in treatment 3 have suffered boron deficiencies, an essential element for plantain production. Despite this, preliminary results are encouraging. Therefore, TNF can be considered as an alternative to mineral fertilization applied to soil for smallholders, under the condition to confirm these results on a damaged soil with different levels of TNF (0.5L to 2L/ha).

P69 - Influence of potassium nutrition and water deficit on leaf gas exchange of banana plants

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An adequate water supply is a critical factor for producing high quality and high-yielding bananas. Although Malaysia receives high rainfall throughout the year, frequent water shortages occur from time to time in some areas. High levels of potassium seem to counter-balance many of the negative effects of water deficit via its role in stomatal regulation[1], osmotic adjustment and turgor generation in the vacuoles[2]. The beneficial role of K suggests that increasing supply to the plant might mitigate the adverse effect of water shortage.

Leaf gas exchange (LGE) of 'Berangan' grown in soils supplied with 50, 300 and 600 g K_2O/plant under irrigated conditions (regular watering), drought (7 days drying cycle) and re-irrigation after a 7-day drought. A 3x2 factorial randomized complete block design with four replications was used to study the interactions with stomatal conductance (Gs), photosynthetic assimilation rate (Pn), transpiration (Tr).

The decline in soil water potential significantly affected LGE in all the treatments compared to the irrigated plants. Withholding water for 7 days decreased soil water potential to -59 KPa. In K-deficit treatments (50 g K_2O/plant) Gs, Pn and Tr were reduced from 285.11±2.48 mmol m^{-2} s^{-1}, 9.98±0.05 µmol m^{-2} s^{-1} and 3.62±0.04 mmol m^{-2} $s^{-1}$to 67.19±0.624 mmol m^{-2} s^{-1}, 3.14±0.04 µmol m^{-2} s^{-1} and 1.16±0.01 mmol m^{-2} s^{-1} respectively, compared to irrigated plants. This is indicative of 76, 58 and 67% reduction in Gs, Pn and Tr, respectively. Water deficit also reduced Gs, Pn and Tr rate of plants supplied with optimum level of K (300 g K_2O/plant) from 387.81±5.24 mmol m^{-2} s^{-1}, 11.87±0.07 µmol m^{-2} s^{-1} and 4.76±0.95 mmol m^{-2} s^{-1} respectively to 140.11±0.48 mmol m^{-2} s^{-1}, 6.87±0.05 µmol m^{-2} s^{-1} and 2.05±0.03 mmol m^{-2} s^{-1} (64%, 42% and 57% reduction in Gs, Pn and Tr, respectively, compared to irrigated plants with optimum K). Stomatal conductance, Pn and Tr in high supplied K treatments declined from 433.23±4.28 mmol m^{-2} s^{-1}, 12.55±0.12 µmol m^{-2} s^{-1} and 2.52±0.02 mmol m^{-2} s^{-1} to 202.28±0.98 mmol m^{-2} s^{-1}, 7.83±0.03 µmol m^{-2} s^{-1} and 2.52±0.02 mmol m^{-2} s^{-1}, respectively compared to irrigated plants (53%, 37% and 49% reduction). No significant difference was observed between high and moderate K treatments with respect to LGE till the third day of water deficit when the soil water potential declined to -27 KPa. On the 7th day of soil water deficit at -59 KPa, plants with high level of K had significantly higher rates of Gs, Pn and Tr (30, 12 and 18%, respectively) compared to plants supplied with moderate level of K. High supply of K partially protects photosynthesis against the deleterious effects of water deficit. Raising the extra chloroplastic K^+ concentration in plant cells by supplying the plant with excess K^+ could possibly reduce water stress inhibition of photosynthesis through the mechanism of a K^+/H^+ antiport system[4]. In irrigated treatments, plants supplied with more K maintained high rate of Gs, Pn, and Tr, (433.23±4.29 mmol m^{-2} s^{-1}, 12.55±0.12 µmol m^{-2} s^{-1} and 4.97±0.03 mmol m^{-2} s^{-1}, respectively) compared to K-optimum (387.81±5.24 mmol m^{-2} s^{-1}, 11.87±0.06 µmol m^{-2} s^{-1}, 4.75±0.95 mmol m^{-2} s^{-1}) and K-deficient (285.12±2.48 mmol/m^2/s, 9.98±0.50 µmol m^{-2} s^{-1} and 3.62±0.03 mmol m^{-2} s^{-1}). This corresponded to an increase of 10.5, 5.36, 4.44%, compared to moderate and 34.19, 20.42 and 27%, compared to K deficient treatment in Gs, Pn and Tr, respectively.

Plants with K-deficiency had lower values of LGE; both in irrigated as well as in drought treatments but the reduction were more severe in water deficit treatments. Plants supplied with higher level of K attained faster recovery for Pn (80%) compared to moderate (64.26%) and low K (49.76%) 24 hours after re-irrigation. High level of K seemed to benefit Pn under water deficit. In intact wheat plants the decrease in photosynthesis during water deficit was less severe at high K supply[3]. The results indicate that K could provide a feasible tool for improving the gas exchange regulation of banana under normal and more specifically in water deficit conditions and enhance faster recovery after re-hydration. The first sign of water deficit in plants was reduction of Gs, followed by transpiration and photosynthesis. The reduction in

Gs consequently reduced Pn and Tr. Similarly, after rehydration Gs recovered earlier than Pn or Tr. Stomatal conductance is a better indicator of water deficit than Pn and Tr.

1. Pier P.A. and G.A. Berkowitz. 1987. Modulation of water stress effects on photosynthesis by altered leaf K^+. Plant Physiol. 85:655-661.

2. Mengel K. and W.W. Arneke. 1982. Effect of potassium on the water potential, the pressure potential, the osmotic potential and cell elongation in leaves of *Phaseolus vulgaris*. Plant Physiol. 54:402-408.

3. Sen Gupta A., G. Berkowitz, and P.A. Pier. 1989. Maintenance of photosynthesis at low leaf water potential in wheat. Plant Physiol. 89:1358-1365.

P70 - Influence of graded levels of nutrients on the growth and yield of tissue culture cv. 'Robusta'

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Although there are extensive information on the nutrition of suckers, very little work has been done with plantlets obtained through tissue culture, which require more fertilizers than suckers, which have a well developed root system. The present investigation was carried out in randomized block design with ten treatments, which consisted of three levels of nitrogen (100, 150 and 200 g/plant) with phosphorus being constant (60 g per plant). The fertilizers were applied in three splits at 90, 150 and 210 days after planting as recommended. The treatments were replicated thrice. Observations on morphological characters, yield and yield components were recorded.

There was a positive response of plant growth by tissue culture plantlets, in terms of plant height and girth to nutrient application. Nitrogen at 200 g per plant and potassium at 400 g per plant resulted in vigorous plant growth and was on par with 200 g of nitrogen and 300 g of potassium per plant. The interval between the production of successive leaves was shorter and more number of leaves with higher leaf area were produced, when nitrogen was applied at 200 g per plant with combination of higher levels of potassium (300 g and 400 g per plant). The initiation of flowers was earlier by 24 and 32 days in the plants, which received higher levels of nitrogen and potassium (200:400 and 200:300 g per plant, respectively). Further, the fruits matured earlier, thus reducing the total crop duration from planting to harvest by 35 days and 31 days, respectively. Higher levels of nutrient application resulted in the production of bunches with greater weights, which can be attributed to the production of more hands and fingers per bunch with higher length, girth and weight of fingers. The quality of fruits, in terms of higher total soluble solids, total sugars, sugar acid ratio and low acidity was improved due to the higher nutrient application (nitrogen at 200 g and potassium at 300 g per plant along with phosphorus at 60 g per plant).

P71 - The nutrient limited factors for yield and quality on banana production in Guangxi

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Guangxi, located below latitude 23° is suitable to grow various tropical fruits, especially banana. In the past few years, banana production has greatly developed, reaching 60 000 hectares in 1999. Due to poor inputs and extensive cultivation and management, yield is only 18 to 20 tons/ha and quality is low. Banana plants require potassium, more than any other nutrient, regardless of its development phase. In general, yield is between 1600 to 4000 kg per hectare whereas the quantity of potassium (K_2O) absorbed by bananas is between 2863 to 19 667 kg per hectare.

According to the results of 12 field experiments, yield was positively correlated with absorbed potassium (r=0.915**). For banana grown on sandy loam soils, the critical soil K value was 76.8 mg/kg of available K. The K supply classes established were: less than 76.8, 76.8-155 and more than155 mg/kg, corresponding to low, medium and high K supply, respectively. In most banana growing region, the available potassium in the soil was less than 155 mg/kg. Applying fertilizer between 477 and 954 kg/ha increased yield by 32 and 66%. When the quantity applied was 1184 kg/ha, the benefit decreased.

P72 - Aluminium toxicity induces lipid peroxidation and affects antioxidant enzyme activities in cultivars of *Musa* sp.

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Response of the antioxidant system to aluminium toxicity was studied in the leaves of 'Berangan' (AA) and 'Novaria' (AAA). Changes in catalase, ascorbate peroxidase (APx) and gluthatione reductase (GR) specific activities in plantlets treated with aluminium at 50 and 200 µM) were recorded at 0, 4, 8 and 10 days. The extent of membrane damage, measured from levels of lipid peroxidation was also examined. In both cultivars, activities of the antioxidant enzymes were significantly higher than the controls. These results suggest that the antioxidant enzymes can be activated in response to oxidative stress induced by aluminium. In 'Novaria', level of lipid peroxidation was lower but activities of GR and APx were higher than that in 'Berangan'. However, Catalase specific activities were remarkably higher in 'Berangan' compared to 'Novaria'. These results suggest that 'Novaria' may have a better protection mechanism against oxidative damage induced by aluminium toxicity by maintaining higher inherent and induced activities of antioxidant enzymes compared to 'Berangan'.

P73 - Response of tissue cultured banana cv. 'Robusta' (AAA) to varying levels of N, P and K

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In recent times, there has been interest in the use of *in vitro* produced planting material. Reports point to the superior performance of tissue culture plantlets, compared to suckers[1]. Banana requires high levels of nutrients for proper growth and production. It is estimated that a crop of fifty tonnes in one hectare removes 320 kg of N, 32 kg of P_2O_5 and 925 kg of K_2O every year[2]. Very little work has been done on the growth dynamics, culture and nutrition of banana plants obtained through tissue culture. Hence, a field trial was conducted during 2000-2002 in College Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-3 to standardize fertilizer requirement for tissue culture plants of. 'Robusta' (AAA) both in the motherplant and ratoon crops. There were six treatments replicated four times in a randomized block design. The treatments consisted of three levels of application: 100%, 150% and 200% of recommended NPK (110:35:330 g per plant) applied in three and four splits (3, 5 and 7 months after planting and 2, 4, 6 and 8 months after planting).

Application of 165:52.5:495 g of NPK per plant in four splits (150% of the recommended dose) resulted in vigorous plant growth with higher girth, more leaves and greater leaf area. Days taken from planting to shooting in plant crop, (ratooning to shooting in case the of ratoon crop), shooting to harvest and total duration were also significantly reduced and bunch weight increased.

1. Robinson, J.C. 1990. A field comparison of conventional suckers with *in vitro* derived banana planting material in the first crop cycle. Acta Hort. 275:181-186.

2. Lahav, E. and D.W. Turner. 1983. Banana nutrition. Int. Potash Inst. Bull. 7:33.

P74 - Effect of time and dosage of paclobutrazol (PP_{333}) on growth and flowering of 'Poovan' banana

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Many commercial cultivars of banana produce large sized plants which are difficult to manage and prone to wind damage. The economic advantage and importance of growing dwarf or semi dwarf varieties has been recognized in most cyclone-affected banana growing areas. With banana, the ultimate method of tree size control is the use of growth retardants[1]. Among the growth retardants, paclobutrazol (PP_{333}), a triazole, was found highly promising for many horticultural crops[1], including greenhouse grown 'Grande naine'[2]. Hence, a study was conducted to standardize the dosage, and time of application of paclobutrazol on growth and yield of 'Poovan' (Mysore, AAB).

The study was conducted at the orchards of Annamalai University in Tamil Nadu, India. The experiment was laid out in a factorial randomized block design with nine treatments, including a control, applied 3 or 5 months after planting. The commercially available form of paclobutrazol, CULTAR (25% w/v SC), was used. Observations were recorded on various growth characters from four tagged plants[3].

Application of PP_{333} 3 months after planting reduced plant height by 24%, leaf area by 13%, leaf length to breadth ratio, petiole length by 22%, sucker production by 12% and total number of leaves, but increased plant girth, number of functionnal leaves, total leaf area, leaf area index and phyllochron. The early treated plants shooted 8 days earlier and matured 10 days) before the late treated plants (5 months after planting). The plants exhibited a dose dependent response. Application of more than 1 ml of active ingredients per plant had severe and undesirable effects.

1. Miller A.N. and D. Swietlik. 1986. Growth and fruiting response of deciduous fruit trees treated with paclobutrazol. Acta Hort. 179:563-566.

2. El- Otmani M., N. Cheikh and M. Sedki .1992. Effect of paclobutrazol on greenhouse-grown banana in Morocco. Scientist Hort. 49:255-266.

P75 - Genotypic variability in root traits and shoot-root ratio of *Musa* spp. Implications to the improvement of the root system

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One way of overcoming banana production constraints lies in the genetic improvement of the root system. However, this requires in-depth knowledge of genetic variability in root parameters. The present study was carried out at the Kawanda Agricultural Research Institute in Central Uganda and assessed variability in root system size and shoot-root dry weight ratio of a wide range of *Musa* spp. genotypes, including 6 wild diploids, 10 East African highland bananas (AAA), 5 newly introduced triploids and 4 tetraploids. All genotypes were assessed for shoot and root growth at 20 weeks after planting, while a subset of five highland bananas was also assessed at flowering. There was a significant effect of the genotype on root and shoot traits of plants/mats at both growth stages. For example, 'Enyeru', 'Nakinyika' and 'Kisansa' had the largest mat root system size 20 weeks after planting. In contrast, the genotypic effect on the shoot-root ratio of the mat was not significant ($p>0.05$) indicating that all genotypes had a similar partitioning of dry matter between the shoot and the root system. In addition, large numbers of significant ($p<0.05$) positive correlations were found between shoot and root traits for mats assessed 20 weeks after planting and at flowering. The shoot-root ratio and correlation results are in accordance with earlier observations made at the IITA high rainfall station on a wide range of genotypes including West African plantains and plantain hybrids (AA, AAA and ABB) and tetraploid hybrids. This implies that changing the shoot-root ratio through breeding may be cumbersome.

P76 – Comparison of root and shoot development in Enset and an East African highland banana

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The *Musaceae* family is subdivided into the genera *Musa* and *Ensete*. While the banana fruit is known worldwide, Enset (*Ensete ventricosum*) is only cultivated in Ethiopia where products processed from the corm and the pseudostem are a major source of food for more than 12 million people. Whereas the banana fruit is harvested less than two years after planting, Enset plants are processed at 5-7 years after planting. The production of both crops is constrained by a number of factors including pests and diseases and a reduced soil fertility. Enset, being monocarpic and thus unbranched, is firmly anchored and toppling of field-grown plants is rare. The aim of this study was to compare root, corm and shoot growth of field-established Enset and East African highland bananas (cultivars 'Siira' and 'Entaragaza') (AAA).

The plants were established at the Makerere University Agricultural Research Institute Kabanyolo in Central Uganda. Growth traits of all the Enset plants and banana mats (i.e. mother plant and lateral shoots) were recorded at flowering of the banana plant crop (1.3 years after planting). The results indicate that Enset had a significantly ($p<0.05$) shorter and thicker pseudostem compared to banana. In addition, Enset had a larger root system with thicker cord roots. Results further show that the Enset plants had a significantly smaller corm and a lower shoot-root dry weight ratio compared to bananas. The lower shoot-root ratio indicates that young Enset plants partition more assimilates towards root growth compared to mature bananas. This leads to enhanced plant stability. In addition, the thick cord roots (>1 cm diameter) of the Enset plants may increase the plant's tolerance to nematodes and anchorage strength. Additional data on shoot and root traits of ready-to-harvest 5 to 7-year-old Enset plants is currently being collected on farm in Ethiopia.

P77 - Review of research on relationships between root traits, shoot traits and bunch weight

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The first studies assessing the relationships between shoot and root traits were predominantly confined to dessert bananas (AAA group) and to sucker-derived plants. Positive relationships were established between the number of cord roots and shoot traits in the dessert banana cultivars 'Poyo' and 'Giant Cavendish'. Partial suppression of roots adversely affected shoot growth and increased the number of days to flowering in 'Giant Cavendish' under aeroponic conditions, whereas linear relationships between shoot and root growth were observed under hydroponic conditions. Field studies carried out at the IITA Onne high rainfall station, Nigeria, revealed large numbers of significant positive correlations between shoot and root traits in 27 *Musa* spp. genotypes belonging to 12 genomic groups and 3 ploidy levels. These relationships were valid for both sucker and *in vitro*-derived plants. In addition, significant positive correlations were observed between shoot, root and bunch weight for the plantain ‘Mbi Egome’ (AAB).

Subsequent studies carried out in Uganda (Kawanda Agricultural Research Institute and Makerere University Agricultural Research Institute Kabanyolo) also found significant and positive relationships between root, corm and aerial growth traits of complete mats in East African Highland bananas (AAA) during the vegetative and early reproductive stage. Additional on-farm and on-station studies focusing on the highland bananas `Mpologoma´, `Lwadungu´, `Nakitembe´, `Mbwazirume´, and `Kibuzi´, the dessert banana `Sukali ndizi´ (AAB), the plantain `Gonja´ (AAB) and the beer banana `Kayinja´ (ABB) revealed strong relationships between bunch weight, root, corm and aerial growth traits. Hence, poor root development will adversely affect shoot and leaf canopy development and as a result reduce yield. The opposite is also expected, i.e. when leaves are affected by black leaf streak disease, the root system is reduced.

P78 - Growth and yield response of tissue cultured banana cv. 'Robusta' to split application of nutrients

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Banana is one of the important remunerative fruit crops in International trade and is grown in all the tropical and non-tropical regions of the world. As bananas are parthenocarpic and seed sterile, multiplication is vegetative by means of suckers. This method, however, has disadvantages such as bulky suckers that are difficult to transport, a very low multiplication rate and is a source of inoculum for many important diseases. These problems can be overcome, to an extent, by *in vitro* propagation, which increase yields and shorten the production cycle compared to suckers. In recent times, the usage of *in vitro* plantlets has gained in importance. It is expected that these *in vitro* plantlets will vary in their nutritional demands because of their accelerated growth rate. This necessitated a detailed study on the nutritional requirements of *in vitro* propagated 'Robusta' plantlets. The experiment was carried out in a randomized block design with seven treatments in three replications. The treatments consisted of application of fertilizers (200 g nitrogen: 60 g phosphorus: 300 g potassium per plant) in six splits at 30-day intervals, seven splits at 30-day interval, eight splits at 45-day intervals, four splits at 60-day intervals, three splits at 75-day intervals and three splits as recommended (90, 150 and 210 days respectively).

Application of nutrients at 30-day intervals in seven splits increased plant height and girth, number of functional leaves at shooting, total number of leaves and leaf area. The crop duration was reduced by 31 days due to earliness in flowering by 23 days. Higher uptake of nutrients resulted in better dry matter accumulation, which was reflected in the highest bunch weight (43 kg) due to the production of more number of hands and fingers per bunch (11.05 and 218.67 respectively). Split application of nutrients (seven splits at 30-day intervals) also produced superior quality fruits with high total soluble solids, total sugars, sugar: acid ratio and low acidity. The present investigation has revealed that the application of 200g nitrogen, 60 g phosphorus and 300 g potassium per plant in seven splits at 30-day interval was optimal for obtaining higher yields.

P79 - Lab to land productivity enhancement in banana by tissue culture

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In recent years there is an upsurge in the interest in biotechnology, which has tremendous potential in solving basic problems of food, fibre, fuel and medicine particularly in Asian countries. Banana is cultivated on more than 48 000 ha in and around Jalgaon district of Maharashtra constituting 71% of the total area of the state. Envisaging an enormous potential for high yielding superior quality planting material 'Jain Group' ventured into a state-of-the-art tissue culture laboratory in 1994 to produce 'Grand naine'. In 1994-95 only 50 000 plants were produced and but demand increased to the point where the lab produced more than 5 m plants in 2003-04.

Cost effective technology can improve continuously the productivity, profitability, stability and sustainability of the farming system. Tissue culture plantlets are disease free, high yielding (28-30 kg bunch in optimal cultural practices compared to 15 kg by using suckers) and accelerate growth, leading to bunch harvest in 11 months compared to 15 months with suckers. Selection of elite plants from mother nursery, virus indexing, utilization of sword suckers for initiation in the lab, control of contamination, genetic homogeneity, conducive microclimatic conditions in growth rooms, extreme hygienic conditions during weaning, supply of fully hardened plants and ultimately better performance in the field have led to a very high demand of Jain tissue culture plantlets not only in Maharashtra but in other neighbouring states such as Madhya Pradesh, Gujrat, Karnataka, Andhra Pradesh, Uttar Pradesh and Chhatisgarh.

Field performance in terms of well developed root system for better absorption, greater functional leaf area leading to high photosynthetic rate and higher yield per unit area of land in a short time is now well established enabling better acceptability among growers. Jain tissue culture unit is the biggest producer of banana in India, providing comprehensive agronomic services and training as well to the farmers. The unit as a whole has been recommended for ISO 9001-2000 certification by TUV, Germany in February 2004. Jain Irrigation Systems Ltd. has pioneered the introduction of hi-tech inputs like micro irrigation system, fertigation, crop geometry, intercropping and ratooning contributing to maximum to improve the smallholder livelihoods through better economic returns.

P80 - Morphological and physiological responses of ratoon crop of banana cv. 'Dwarf Cavendish' (AAA) to bioregulators

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In the present study, the physiological and biochemical responses of the ratoon crop of 'Dwarf Cavendish' (AAA) to biological derivatives of sea weeds and microorganisms such as plantozyme and biozyme were assessed. The experiments conducted during the year 2002-2003 had seven treatments, including the control T1 (recommended doses of $N:P_2O_5:K_2O$ of 200:40:200 g per plant per year), T2 (soil drenched with plantozyme 0.2% at the time of setting the suckers, T3 (soil drenched with biozyme 0.2% at the time of setting the suckers), T4 (10 gram of granular plantozyme per plant 2 and 4 months after setting), T5 (10 g of granular biozyme per plant 2 and 4 months after setting), T6 (foliar spray of plantozyme 0.2% 6 and 8 months after setting), T7 (foliar spray of biozyme 0.2% 6 and 8 months after setting. The physiological and biochemical changes and agronomic performance of the plants were recorded at shooting.

Plant height, pseudostem girth and leaf area index were significantly influenced by the foliar spray of plantozyme 0.2% (Table 1). The treatment resulted in increased pseudostem height and girth. The increased height coupled with girth revealed the influence of nutrients and amino acids in plantozyme on higher cell wall plasticity and dry matter accumulation. The ecophysiological influence was also witnessed with higher leaf area index and lower light transmission ratio (LTR). The lower LTR and higher canopy coverage favoured the plant for better light interception and its use efficiency. Although leaf chlorophyll content registered a higher value, the chlorophyll stability index showed no significant difference between treatments.

Net photosynthesis (*Pn*) measurements were taken using CI 301 PS CO_2 analyser (CID Inc, USA)[1]. Plants treated with foliar spray of plantozyme 0.2% showed higher *Pn* in the third youngest leaf. Stomatal conductance (g_s) also showed the same trend The increased *Pn* indicates more dry matter production and that was well evidenced through improved pseudostem height and girth with low LTR. The better growth and development of the plant was also observed through higher transpiration (*E*), which in a previous study has been reported as 16.4 μmol m^{-2} s^{-1} under normal conditions[1]. Higher *E* is essentially required for more gas exchange resulting in maximum *Pn* and g_s *E*. The values recorded in the present study are low but comparable to the ones from another study[2]. The photochemical efficiency of chlorophyll in terms of ratio of variable fluorescence (*Fv*) to maximal fluorescence (*Fm*) was measured using Plant Efficiency Analyser (Hansatech, UK). The maximum *Fv/Fm* was registered in plants treated with foliar spray of plantozyme 0.2% during sixth and eighth month after setting. The influence of bioregulators on auxin content was also assessed by estimating IAA oxidase activity. Among the treatments, foliar spray of plantozyme 0.2%resulted in lowest IAA oxidase activity indicating higher levels of unoxidised auxin.

Analysis of leaf nutrient status showed significant variations in nitrogen (N) and potassium (K) contents. Plantozyme 0.2% improved the leaf N and K considerably (Table 2). However, phosphorus (P) showed no significant change. The increased leaf N and higher nitrate reductase (*NR*) helped in efficient N assimilation for better growth of the plant. The influence of bioregulator on growth characteristics and physiological attributes resulted in significant yield improvement. The foliar spray of plantozyme 0.2% during sixth and eighth months after setting registered higher bunch weight. The maximum dry matter production and its partitioning due to higher *Pn*, g_s, *E* and leaf nutrients (N and K), *NR* and, lower LTR and IAA oxidase activity helped the plant to have better growth and yield. The fruit quality attributes such as total sugars and total soluble solids (TSS) were significantly influenced by the bioregulators. Plantozyme 0.2% resulted in higher sugar content and TSS due to efficient translocation of available photosynthates to fruits and hydrolysis of complex polysaccharides into simple sugars.

1. Eckstein K. and J.C. Robinson. 1995. Physiological responses of banana (*Musa* AAA: Cavendish subgroup) in the tropics. I. Influence of internal plant factors on gas exchange of leaves. J. Hort. Sci. 70:147-156.

2. Jeyakumar P. and N.Kumar.2002. Bioregulators for increased fertiliser use efficiency in banana cv. Dwarf Cavendish (AAA). Pp. 70-82 *in* Proc. 13th International Fertiliser Symposium: Fertilisers in context with resource management in agriculture, June 10-13, Tokat, Turkey.

Table 1. Effect of bioregulators on the performance of 'Dwarf Cavendish'.

Treatments	Pseudostem height (cm)	Pseudostem girth (cm)	Leaf area index	Pn (µmol m^{-2} s^{-1})	E (µmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	Fv/Fm	LTR (%)
T1	148.4	49.1	2.40	20.4	5.22	185	0.794	51.4
T2	165.3	54.3	2.94	24.5	6.85	210	0.814	40.2
T3	160.4	53.1	2.87	23.2	6.30	206	0810	42.4
T4	155.6	50.2	2.72	22.7	6.01	201	0.808	48.1
T5	151.5	49.4	2.61	22.0	5.84	197	0.803	49.5
T6	171.2	58.4	3.41	26.3	8.45	228	0.865	32.7
T7	168.4	56.3	3.02	24.8	7.68	215	0.832	36.4
CD (0.05)	4.80	2.16	0.62	1.80	0.64	8.9	0.051	6.46

Table 2. Effect of bioregulators on the nutritional status of 'Dwarf Cavendish'.

Treatments	N (%)	K (%)	NR (µg NO_2 g^{-1} h^{-1})	IAA oxidase (mg g^{-1} h^{-1})	Bunch weight (kg)	Total sugars (%)	TSS (%)
T1	1.72	3.11	7.52	0.48	18.0	14.0	19.7
T2	2.01	3.65	8.78	0.68	21.7	15.1	24.0
T3	1.98	3.54	8.36	0.63	19.6	15.0	23.8
T4	1.89	3.38	8.21	0.57	19.3	14.7	21.2
T5	1.75	3.24	8.14	0.52	19.1	14.2	20.7
T6	2.07	3.92	9.63	0.74	26.3	16.6	25.7
T7	2.04	3.84	9.08	0.70	24.0	15.5	24.1
CD(0.05)	0.038	0.064	1.18	0.062	1.56	0.54	1.22

P81 - Sustainable crop management practices in bananas – turning eco-bananas a brighter shade of green

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There has been increasing interest in the past five years in the development of banana production systems, which have less impact on the environment. Some of these systems are accredited by organizations such as the Rainforest Alliance as meeting a particular set of standards. However, much research and development is still required to verify that these farming systems are achieving their aims, and to develop new techniques/practices for these systems.

The following research and development areas need particular attention:

- ✓ Understanding the relationships between crop nutrition and pest/disease incidence
- ✓ Understanding the interactions between soil organisms and the application of agrochemicals and soil physicochemical characteristics
- ✓ Investigation of intercropping, polyculture, agroforestry and mixed variety plantings
- ✓ Alternatives to herbicides for weed control
- ✓ Alternatives to fungicides for leaf disease control
- ✓ Rationalization of land-use choices
- ✓ Investigation of slow-release fertilizers and their application to further reduce losses to the environment
- ✓ Cost/benefit analysis of new practices or systems.

For organic systems greater investment is also required in improving the efficiencies of production. Research on organics is what I call ‘space research’ as breakthroughs in this area will have many spinoffs for more conventional production systems. We must also begin engaging with exponents of Biological Agriculture working together where possible in the development of more sustainable production systems.

Regarding drivers of change – ideally this should come from the marketplace. Farmers/land managers will then be more interested in making changes provided it is feasible. However, most studies indicate that the issue of the environment is not amongst the most important issues affecting the purchase decisions of consumers. Rather they are price, appearance and taste. Thus attention to these latter issues coupled with the environment (e.g. do bananas grown with much less fertiliser taste better?) should help in providing pull from the marketplace. If change towards more environmentally sustainable production practices is not proceeding quickly enough then governments must consider incentive schemes such as tax breaks and so forth to act as catalysts in the change process.

For meaningful change in environmental sustainability to occur then adoption of new or improved practices and systems by land managers is the key. Therefore any process for R, D&E must include land managers. Key principles to consider for this process are:

- ✓ It needs to be flexible to cater for the different needs of different farmers and different groups
- ✓ Look at what farmers are doing already and build constructively on this
- ✓ Some practices and innovations are adopted more readily – characteristics like responding to a need, making an observable difference, or demonstrating a measurable benefit in line with farm objectives
- ✓ Farmers need to be able to adapt sustainable practices for local conditions
- ✓ Adoption of new practices is a continuous process and occurs through a number of avenues
- ✓ ‘Real’ participation of farmers and stakeholders is a key.

The process to improve the sustainability of production systems needs to consider all these points within the context of economic, social and environmental sustainability.

P82 - Cultivation of bananas under greenhouse

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Protected cultivation of tropical fruits is gaining in importance all over the world for reasons including control of pests and diseases, increase of yield, improvement of quality and saving water. The banana is the most extensively tropical crop planted under greenhouse; Canary Islands and Morocco have more than 3000 ha under cover and greenhouse banana plantings are also increasing in Israel.

The main reasons for cultivation of banana under greenhouse includes: higher yield, improved quality, better banana cycling and consequently the possibility of timing the crop for the months of better prices, reduction of water consumption and easier control of pests and diseases. The above reasons make banana production more predictable. This paper illustrates techniques of greenhouse cultivation, different types of covers and environmental changes derived from cultivation under greenhouse as well as their influence in banana growth, phenology and production.

P83 - Improvement of livelihood of banana farmers in the Sultanate of Oman

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In Oman banana cultivation has been practiced since pre-Islamic time onwards. The farmers mainly cultivated the local variety 'Melindi' (AAB Group) as a perennial crop. They mainly cultivated the banana in a organic way without applying any inorganic fertilizers and pesticides. M/S. Al-Batinah International (SAOG) company has been formed to procure the bananas from small farmers in the Batinah region of Oman with the aim of improving their livelihoods. As a result, the farmers are selling their bananas at a fair price that encourages other farmers to grow bananas in smallholdings. The company provides technical advice to farmers to improve the production. In the near future, the Batinah region will become one of the most important exporter of bananas in Oman and in the entire Middle-East.

P141 - Organic plantain production for niche markets in central Kerala, India

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The move from conventional to organic production of bananas involves a substantial change in crop production methods. In the Indian subcontinent, Kerala state has unique organic banana systems[1]. This paper outlines a fully organic plantain production system, using the clone French plantain var. Nendran clone Chengalikodan liked for its high productivity, catering exclusively to the festival markets of central Kerala.

Planting is in 1 m deep furrows, giving wide space between plants. Heavy doses of organic manures are added at the time of planting and subsequently at frequent intervals. The organic amendments include farmyard manure, green leaves, wood ash, neem cake and other oil cakes like groundnut cake. Irrigation is given on alternate days, withholding occasionally to prevent the development of white shoot, a common physiological disorder. Pest and diseases are managed by cultural and other ecofriendly methods.

Other features of this system are the unique bunch management practices, which include cyclic rotation of the bunch at shooting, so that the first hand faces away from the pseudostem, covering the bunches with thick layers of dry banana leaves, and giving support to each hand by inserting thick pads of dry leaves. Initial wrapping is done 28 days after bunch emergence and redone one month later. Before wrapping, the fruits are wiped carefully with moist cloth to remove dust and dirt.

Propping with two props is done to support the heavy bunches. The bunches are uniformly golden yellow at maturity and mean per plant yield ranges 25–30 kg compared to 10–12 kg under normal production systems. These fancy bunches fetch 4 to 5 times more than the normal price for this variety. This production system mainly focusses on producing bunches for offering at places of worship and to give as gifts during the state festival.

1. Suma A., Jyothi Bhaskar, Rema Menon, K Anita Cherian and P. Prameela. 2002. Bunch bagging in French plantain var. Nendran (*Musa* AAB). Pp. 90 *in* Global Conference on Banana and Plantain, Bangalore, 28 – 31 October 2002.

Session 4

Post harvest and processing for the diversification of income. Innovation and uptake

Oral presentations

Keynote: Farmer learning and agro-ecological and crop pest management of plantains and bananas in Nicaragua

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Limited-resource farmers throughout Nicaragua cultivate various *Musa* with minimal expenditures for inputs and labour. This low risk strategy can be complicated by inadequate management of pests and diseases leading to reduced bunch size, plant loss and fewer production cycles. Commercial pesticides are both ineffective and beyond the financial reach of most farmers. Can more timely pest management strategies be developed consistent with the low risk-low input strategy that makes *Musa* production a useful commercial and home consumption complement in rural livelihoods? A NORAD-financed project run by CATIE has collaborated with universities, government agencies, NGO's and farmer groups to develop a participatory group learning approach by crop stage for agro-ecological pest and crop management. The approach incorporates: farmer experimentation; learning exercises which strengthen farmer ecological knowledge of the crop, other primary producers, pests and beneficial organisms, and soil factors; simple scouting methods for farmer observation of crop, pest, and system indicators; and group discussions at key moments in the crop cycle.

In the first meeting prior to crop planting, farmers discuss pest and crop problems and their experience with different practices, prioritize critical problems and possible alternative practices for testing, and identify indicators for evaluating the group's progress. Farmer volunteers establish experiments and complete scouting procedures in their fields and report back at later meetings. In each of the 4-5 meetings over a year-long cycle, farmers analyze recently planted fields and fields in first, second and more crop cycles. In this way, the multi-year crop can be studied during a single year.

From 1999 through 2001, MusaNic, the Nicaraguan multi-institutional *Musa* training and research group, of which the CATIE team was also a participant, trained over 90 field technicians from 36 organizations working with more than 1100 farmers. CATIE and its collaborators documented the approach in Spanish on a compact disc that is available for other projects and organizations working in farmer and field technician training in *Musa*. The CD has four sections: banana and plantain production zones in Nicaragua; the growth and crop stages of the *Musa* plant; the ecology and management of the *Musa* food web and agro-ecosystem; and how to strengthen farmer reasoning to manage the *Musa* agro-ecosystem food web for low-risk, low-cost increased crop production. Each section is illustrated with photos and figures. The section on crop and pest agroecology presents information pest by pest and by crop stage on the climatic and biological factors that favor or suppress pests as a basis for planning management practices. The section on strengthening farmer reasoning contains workbooks, scouting methods, learning exercises, guides for farmer meetings and practical experiments as resources for field technicians working with farmers. Simple tools for evaluating farmer knowledge are also illustrated.

Keynote lecture: Partnerships and networking in the tropical fruit industry: the experience of the International Tropical Fruits Network

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There are sufficient resources, knowledge, wealth and talent available that can be shared with others. Much effort and energy had been mobilised in the past to reduce hunger, malnutrition, poverty and social inequity, obviously much still needs to be done, perhaps in more innovative ways. Many organizations with noble goals have been created at local, national and international levels to improve rural livelihood, income and more importantly to restore human dignity. Some have done well while others have slowly become redundant or even irrelevant.

Like many international organizations, TFNet is established to fulfil a given mandate, in this case, to promote sustainable development of the tropical and sub-tropical fruit sector, in relation to production, consumption, processing, marketing and international trade. In this regard, with the mission to link people, technology and market, TFNet has embarked on a number of collaborative projects, forging strategic alliances with like-minded organisations, especially with its diverse membership consisting of government institutions, NGOs and private sector. Based on this experience, TFNet has identified some key factors that need to be addressed in fostering genuine partnerships among organisations and with the intended rural clientele. These factors include leadership, good governance, transparency, knowledge management, creativity and empowerment.

Banana cultivars in Micronesia: newly recognized sources of provitamin A and total carotenoids and other nutrients

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Banana is eaten as a major staple food in the four states of the Federated States of Micronesia (FSM) and there is a great diversity of banana cultivars there. Yet few studies have been conducted on the nutrient content of FSM bananas despite the fact that micronutrient deficiencies, including vitamin A deficiency (VAD) and anemia, are public health problems. Chronic diseases, including diabetes, heart disease, and cancers, are also serious problems in FSM. The increasing prevalence of these diseases has been related to greater consumption of imported foods. Epidemiological evidence indicates that carotenoid-rich foods may help to prevent VAD and these diseases. It is thus important to identify locally-grown and acceptable nutrient-rich foods, which can be promoted for health improvements. Yellow or orange coloration is a general indicator of carotenoids in foods. A study was conducted to identify carotenoid-rich sources among FSM banana cultivars that may be promoted in an intervention to alleviate nutritional disorders in FSM; to document FSM banana cultivars; and to initiate an intervention for increasing production and consumption of selected banana cultivars.

An ethnographic approach, including key informant interviews, observation, and photography, was used to identify cultivars with potentially high-carotenoid content according to flesh coloration, focusing work in Pohnpei and Kosrae States. Composite samples of ripe raw and cooked bananas were analyzed for β-carotene (the most important provitamin A carotenoid), and other carotenoids and nutrients. Flesh color was measured using the Roche Color Fan. Research was conducted to determine the cultivars presently grown in Pohnpei and their important characteristics. A germplasm collection was initiated.

Carotene levels ranged from 30 to 6360 µg/100 g among the 21 cultivars studied. Fifteen cultivars (including two Fe'i bananas), all well-liked for taste, were identified with high levels of provitamin A and total carotenoids, meeting half to all of estimated dietary requirements for vitamin A intake within normal eating patterns. Nine cultivars had β-carotene levels 25 times the amount in Cavendish banana. Carotenoid levels were greater in bananas of deeper flesh coloration. The carotenoid-rich 'Karat' (a Fe'i banana) is a traditional weaning food in FSM, but has become rare due to neglect since the 1970s, like other FSM carotenoid-rich bananas. 'Karat' had high levels of riboflavin (vitamin B_2), a vitamin that is related to iron metabolism.

A number of FSM banana cultivars have significant carotenoid levels and should be promoted for their health benefits. Further work is needed to identify and promote carotenoid-rich cultivars in FSM, establishing the bioavailability of carotenoids in banana, and further characterization of FSM banana cultivars. Aspects of this work may be important in other countries where banana is a major food.

Improving food and livelihood security among smallholder banana and plantain producers in sub-Saharan Africa

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The push to increase banana and plantain production in sub-Saharan Africa has led to increased yield of these commodities in many countries in the sub-region. But turning the surplus from the increased yield into income and into development is a goal, which is still far from being achieved in many of these countries. The reason for this slow take-off is due to the fact that the use of modern facilities for post-harvest handling including packinghouse operation, CA storage, processing, among others, can not be justified as of now for handling produce from smallholder producers. Organizing smallholders into industrial organizations or getting them to enter into an industrial partnership with large-scale commercial ventures in the sub-region may be one of the possible avenues for solving the post-harvest and marketing problem. In this paper, possible approaches that could lead to viable post-harvest technologies as well as a sustainable improvement in food and livelihood security for rural smallholder banana and plantain producers are examined and discussed.

Processing and food uses of bananas and plantains in Cameroon

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In southern Cameroon, bananas and plantains play an important role in the diet. There are many food uses of these crops relative to the eating habits of consumers. The processing and culinary methods of bananas and plantains including the estimation as well as the measurement of the quantities of ingredients used and the traditional utilisations of these food stuffs in two Cameroonian towns (Bafoussam and Yaoundé) have been investigated and are described in this study. Some culinary preparations using different cultivars are common in these regions such as roasted or fried plantain, plantain chips, boiled plantain or banana and pounded plantain. They are eaten with various sauces, vegetables and other food complements. Other preparations found in those regions include stuffed plantain or banana, plantain or banana porridges and traditional meals called *kondre* and *malaxé*. Preparation of chips, fried and roasted plantain is mainly carried out by women and young boys on the streets to diversify incomes. These various transformations and uses of bananas and plantains contribute to the reduction of post–harvest losses as well as the valorisation of these perishable foodstuffs. The evaluation of their nutrient composition (macro and micronutrients) as well as the effects of culinary preparations and the bioavailability of these nutrients are being conducted at the CARBAP post–harvest technology laboratory in order to appreciate their contribution in the fight against malnutrition in some regions of Cameroon.

Hybrid cooking bananas: a study of taste-panel preferences between clones

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Agronomic and post harvest assessments on the consumer acceptability of the hybrid cooking bananas BITA-3, FHIA-25 and FHIA-03, of 'Saba' and 'Yangambi km 5', and of local cultivars 'Apantu' (False Horn) were conducted in Ghana. Five recipes: chips, *kakro, ofam, ampesi* and fried ripe plantain were prepared. Fifteen untrained male and female tasters were involved. FHIA-25 produced the heaviest bunch (45kg) and the largest number of fingers (220). All the hybrids, except BITA-3, retained relatively high number of functional leaves (6-8) at harvest. When processed, all the hybrids were preferred for *kakro* and *ofam*. 'Saba', 'Yangambi km 5', BITA-3 and FHIA-03 were highly preferred. FHIA-25 and 'Fougamou' were rejected to make *ampesi*, chips and fried plantain. Fruits from the tetraploids tended to be softer than fruits from triploid clones when boiled as *ampesi*.

Plea for a new banana production policy in Côte d'Ivoire

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From 1930 to 2001, the banana sector in Côte d'Ivoire went through poor, uncertain, hesitating or wealthy behavior. All of these situations are analyzed under bibliographical investigations in order to: show how crises have been solved; build knowledge and plan for appropriate development policy; show the Ivorian experience; teach the Ivorian banana sector actors; identify main factors able to influence this important economical sector; Induce research activities focus on the development of the banana sector.

Another outcome of the study is related to research themes thought to improve the Ivorian banana proficiency. On the one hand, the Ivorian experience was built by producers themselves with strong support from the government. Many smallholders have contributed to increasing the production until 1974, but they did not master production techniques and they were poor. As a result, the banana from Côte d'Ivoire was known as one of bad quality until the 1990s. Directives were given to strengthen the sector and support from the European Union was determinant. To improve quality, smallholders have been trained. However, intensification of the culture was gradual. On the other hand, banana availability on the local market is closely related to the quality of the production. Right now, less than 10% of the production is sold in the main towns. The other towns and rural communities (about 9 million consumers) are not supplied with banana (AAA, Cavendish). In addition, several countries in West Africa (Burkina Faso, Guinea, Niger, Senegal, Mali, etc.) depend on the Ivorian banana production. Such a situation threatens food security in West Africa. Therefore, the importance of a new banana production policy becomes evident. With this new policy, research centers' improved varieties will be promoted. The rural consumers will be invited to select the varieties themselves on a participatory selection system. Such a policy can instigate a second banana economy held this time by rural communities.

Effect of bunch covering and postharvest treatments on quality of banana during storage at low temperature

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Proper bunch management technologies, particularly covering bunches with polyethylene bags is a common practice in several banana exporting countries. It has been observed that the bags not only increase bunch size and weight but also affect the post harvest quality of the fruits. In the present investigation an attempt has been made to study the effect of bunch covering, postharvest hot water treatment and ethylene absorbent on shelf life and quality of 'Neypoovan' (AB) during storage at 13.5^{o}C.

Fully mature bunches covered with 6% ventilated polyethylene bags were harvested, dehanded and treated with hot water at 51^{o}C for 17 minutes and sealed in polybags of 150 gauge thickness along with 30 g of ethylene absorbent and stored at 13.5^{o}C. Fruits from uncovered bunches were used as control with and without hot water treatment and ethylene absorbent. The results show that the physiological loss in weight was lowest (1.5%) in covered bunches compared to hot water treatment (2.7%) or absolute control (16%) after 21 days of storage. The moisture content, total soluble solids, total sugars and acidity were lowest and starch content was highest in covered bunches. The scores of colour, flavour and texture were higher in covered bunches compared to control. The green life was 35 days in covered and hot water treated bunches compared to 21 days in control. The yellow life was 4 days for covered bunches compared to 2 days for hot water treated bunches and 1 day in the absolute control. It is concluded that pre-harvest bunch covering coupled with postharvest hot water treatment at 51^{o}C and storing along with ethylene absorbent can enhance the storage life of banana up to 39 days at 13.5^{o}C.

Agro-industry characteristics and marketing chain of banana chips of ‘Agung semeru’ in East Java

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Banana chips made with ‘Agung semeru’ bananas plays an important role in the development of banana agro-industries in Lumajang regency. The aim of this research was to identify the characteristics of the marketing chain as a base of government decision in developing this agro-industry in Lumajang regency. Research was done from September until December 2003 using a survey. Data were collected using a questionnaire answered by farmers, processors and marketing agents chosen at random. The result show that there were many banana chip industries in this regency, most of them small-scale industries that use mostly family members for labour and their house to do their activities. The bananas are supplied by the farmers through wholesalers. There was an interaction between the production subsystem and the marketing and processing subsystems.

Pre-shipment and shipboard factors influencing the out-turn condition of banana cargoes

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Information is obtained during out-turn surveys at destination (on behalf of cargo receivers, underwriters, ship-owners or charterers) or during the study of claims documentation submitted by lawyers acting for one or other of the above parties. There may also be occasion to visit the producer country. Pre-shipment factors influencing cargo quality and out-turn condition include the weather, crop husbandry in relation to leaf spot diseases, harvesting and handling techniques, post-harvest treatments, method of packaging, schedule of loading, and carriage instructions written by the shipper/exporter. Shipboard factors include design and function of the refrigeration and ventilation equipment, method of stowage, interpretation of carriage instructions, and duration of voyage. For container shipments it is the shipper's responsibility to "stuff" the container in an appropriate manner; the container operator accepts the closed box and undertakes to supply refrigeration/ventilation in accordance with shipper's carriage instructions. Case studies will be presented, demonstrating that deterioration (such as premature ripening) is often the result of a combination of adverse factors. Particular problems include the difficulty of achieving uniform air circulation through a palletised stow, and the challenge of shipping additional commodities (such as melons or citrus) in the same vessel. Accurate diagnosis of the causes of deterioration can assist in prompt settlement of claims and reduction of losses in the future.

Banana production systems in East Africa

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Eastern Africa contributes nearly one-quarter of the global banana production. Most of the production is on smallholdings ranging from 0.5 to 3 acres. In today's fast-evolving global economy, these systems are faced with monumental challenges of sustainability. In the region, regarded as a secondary diversity centre of bananas, an estimated 200 to 300 named cultivars are threatened by declining soil fertility, increased pest/disease pressure and socio-economic constraints.

In general, production conditions in the region are no ideal for bananas. Instead the present systems seem to have derived from an interaction between the genotype, the agro-ecological conditions and socio-cultural needs of the communities that grow the crop. What are the limiting factors in these systems? What needs to be done to address them?

The paper provides the latest information on the banana production systems based on spatial patterns with respect to cultivar diversity, agro-ecological and socio economic conditions in as far as these factors affect the productivity of the farming systems. It discusses the strengths and weaknesses of the systems and suggests what needs to be done to address the sustainability challenges that currently face the banana-based production systems in the region.

Technologies developed for postharvest handling of Malaysian bananas

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Banana is one of the priority fruits under the Malaysian National Agriculture Policy. An integrated research program on postharvest handling of bananas has been formulated to develop appropriate technologies to cater the needs of both domestic and export markets. The cultivars studied were 'Mas', 'Embun', 'Berangan' and Cavendish bananas. Relevant technological information has been generated and developed comprehensively on maturity indices, packinghouse operations, packaging, storage, transportation and ripening. The development of modified atmosphere technology has allowed the bananas to be stored for more than eight weeks in combination with refrigeration at 14°C. Carbon dioxide injury was identified to be one of the major obstacles in technology development. The studies were extended to simulation and actual handling trials by sea shipment in refrigerated containers to other countries including Hong Kong, Japan, West Asia and Europe. The trials were conducted by MARDI jointly with other agencies including FAMA, FELCRA, local exporters and importers.

Banana production in India

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Banana is one of the most important fruit crops of India, which contributes about 37 per cent of total fruit production and provides livelihood security to thousands of people. The crop is largely grown by small-scale and subsistence farmers. The farmers in different regions of the country adopt various production systems such as garden cultivation, wetland cultivation and perennial cultivation depending upon the agro climatic conditions. With a total production of 14.2 million tonnes grown on 4.7 million hectares, India is the largest producer of banana in the world, contributing to about 22% to the global production. Concerted efforts through research and development during the past decade have resulted in an appreciable increase in production and productivity of banana in the country. Productivity has increased from 20 tonnes/ha in 1991 to 34 tonnes/ha in 2000. Productivity reaches 65 tonnes/ha in some states such as Maharashtra. A National Research Centre under the Indian Council of Agricultural Research is working exclusively of banana research. The Centre maintains a rich germplasm bank containing 690 accessions.

Due to varied agro-climatic conditions a large number of verities of banana are being cultivated in various parts of the country. Though more than 20 varieties are being cultivated commercially ‘Dwarf Cavendish’ forms the mainstay of the Indian banana industry due to its high yield, market acceptability, short crop cycle and high economic returns per unit area. The other commercial varieties of banana grown are ‘Poovan’, ‘Njali poovan’ and ‘Rastali’. Short distances are by road whereas the longer ones are by rail but no specialized vans or wagons exist for transporting bananas, post-harvest losses are high. Although there is no standard for grading and sorting, size of the bunch and external fruit appearance determine the quality of the produce. Despite being the largest producer of banana in the world, the Indian share in the export market is negligible. Large local consumption, presence of large players in the international market and poor market promotion are some of the reasons for low exports.

With a view to supplement the Governmental efforts in promoting banana cultivation, a FAO-assisted project on improvement of banana production for small-scale growers was launched in December 2002 for a period of 18 months. The project has demonstrated the use of disease-free planting material snf improved production and post-harvest management systems. The demonstration plots in Andhra Pradesh and Maharashtra helped disseminate the technology to a large number of farmers in these States, where productivity levels of over 100 t/ha could be attained. Automated packing facilities have been built in Jalgaon, Maharashtra, the first of its kind in the country. The project has played a catalytic role in inducing many farmers to take up improved production and post-harvest management techniques.

Banana enterprises development in Africa - Opportunities and challenges

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Bananas and plantains are the fourth most important starchy staple after rice, maize and wheat, in terms of gross production tonnage. It is also the fifth most important tropical fruit after mangoes, pineapples, avocados and papaya. The crop thus plays a dual role as a food staple for most of the tropical world but also as a table fruit sold at both local and international markets. An estimated annual production of nearly 100 million metric tonnes is produced globally, nearly 30% of which is produced in Africa on smallholder systems with an acreage ranging from 0.5- 3.0 ha. In the continent, most of what is produced is consumed on farm and any excess may be sold in local urban markets. Annual per-capita consumption in some African banana producing regions is estimated at 400-600 kg, the highest in the world. Ivory Coast, Cameroon, Somalia, Ghana and Cape Verde are the only African countries exporting a combined tonnage of 427 000 tonnes.

Despite the above attributes, the crop in sub-Saharan Africa has not contributed significantly to the alleviation of the ills that besiege the continent. Of the 10-13 million tonnes globally exported, Africa contributes only 3%. Moreover the continent for several decades now has experienced declining productivity and yields in subsistence farms are as low as 4-10 tonnes/ha, which is many times less than the expected 30-40 tonnes/ha from research estimates. The low productivity figures are attributed to poor management technologies as well as weak supportive policies, which in many countries have construed banana/plantain only as a food security crop. Thus banana/plantain in the majority of countries does not enjoy the same policy support that normally provides other crops with infra-structure development and other financial incentives. This has in turn resulted into limited private sector investment on the crop; limited crop processing and value-addition prospects and in many countries a confirmed poor man's crop. Consequently, banana/plantain-based cropping systems in the majority of countries suffer from chronic poverty-related problems.

In response to these challenges, a number of enterprises dealing in a wide range of banana products including planting materials, fibre products, beverages and a wide range of food stuffs have evolved on the continent. It is hoped that these enterprises will inject new energy by creating an economic pull that will reach grass-root producers. This paper provides case study reports on banana-based enterprises in Africa and discusses the opportunities and challenges, the banana industry in Africa is facing.

Banana house: spectrum of banana products

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In Malaysia, bananas are an economically important food crop. The ripe banana is utilized in a multitude of ways in the human diet; eaten raw or served in fruit cups and salads, sandwiches, custards and gelatins, or incorporated into ice cream, bread, cakes, muffins, cream pies and a variety of traditional cakes and local desserts.. Ripe bananas are often sliced lengthwise, baked or broiled and served with a garnish of brown sugar or chopped peanuts. If coconut milk is added, it can be served with glutinous rice. Whole, peeled bananas can be spiced by adding them to a mixture of vinegar, sugar, cloves and cinnamon which has boiled long enough to become thick.

MARDI successfully developed varieties of products from bananas in an attempt to diversify its uses and set up a potential banana-based food industry. The products developed include traditional cakes and dessert, puree, dehydrated flakes, flour, jam, jellies, fried banana plantain and banana chips (salty and sweetened) dehydrated bananas or banana figs, osmo-vac banana, crisps, crackers, sauces (dessert, hot and spicy), frozen breaded banana or ready-to-fry banana fritters , banana drink, banana rolls and balls. Banana puree is important as infant food and can be successfully canned by the addition of ascorbic acid to prevent discolouration. Banana puree can be either frozen, canned or aseptically packed for further use as a base for processing into other processed products. Research is still on going to develop more products such as ready-to-use fillings, cordials, nectar, canned banana slices and starch. A banana house concept will be developed in order to promote the goodness of banana to the public and act as a place where one can purchase fresh banana of all varieties and processed products.

Session 4

Post harvest and processing for the diversification of incomes

Posters

P84 - Moisture level of plant residues used as storage media influenced post harvest behaviour of mature plantains

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Neem leaf powder and rice husks, used singly or mixed in three ratios were moistened to 0, 50 and 100% water holding capacity and were evaluated as storage media for mature green plantain fruits. Fruits lined on laboratory table served as control. Data collected included ripening pattern, weight loss and culinary quality traits. Aspect of physical characteristics of media was determined. Heat of decomposition in moistened media containing high proportion of neem leaf powder blackened fruit peels whereas temperature in moistened ricehusk was slightly less than the ambient. Water holding capacity, bulk density and pore spaces of media were similar, but, moisture retention at 60-cm tension was significantly different (p=0.05). Green-life was 6.7, 8.4 and 11.8 days with rice husks moistened to zero, 50% and 100%, respectively, compared to 18.8 in control. Similarly, complete ripeness stage was attained 11, 12.5, 17.8 and 27.6 days, respectively. Shelf life was 18 days in control but 20.8, 27.0 and 32.1 days for fruits stored in rice husks. After three weeks of storage, control samples had lost about 43% of their original weight while fruits stored in moistened rice husks maintained their weight. Organoleptic tests revealed taste as a major determinant of acceptability of boiled sample.

P85 - Snacks made from banana

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Countries that export fresh banana normally process the surplus or the portion that does not meet export standards. Snacks are among the products developed for local market and export. Products include banana chips (salted and sweetened/glazed), dehydrated and smoked banana (banana figs), banana crackers and crisps. Initial research on snacks used the cooking varieties and one of the dessert cultivars, 'Berangan'. Product development with 'Mas' was carried out when it was identified as the potential variety for export of fresh banana. MARDI varieties MB1 and MB2, which are resistant to Fusarium, were also tested for the production of snacks. The maturity stage of the raw material used, the processing parameters and shelf life of the products processed were determined.

The recommended variety for salted banana chips is 'Tanduk' for its golden yellow colour, taste, texture and product recovery of 36-40%. 'Awak' was also tested. While 'Berangan' and 'Mas' (40-45% recovery) were found suitable for sweetened banana chips, but 'Awak' is normally used by processors. Matured banana are used for producing chips. 'Berangan' and 'Mas' (23% recovery) and 'Kapas' (12% recovery) were tested for developing smoked and dehydrated bananas. Another dehydrated product known as osmo-vac banana (6.6% recovery) was also developed using osmotic dehydration method. Other snacks developed include banana crackers ('Berangan') and banana crisps ('Mas')

Both salted and sweetened banana chips packed in paper aluminium laminate and metalized polyester can be kept for more than 6 months. Products quality is still good, crispy and rancidity is not detected. Dehydrated/smoked banana are classified as intermediate moisture food and packed in polypropylene (0.125mm) can be kept for 6 months.

Proximate analysis indicated that the moisture content of banana chips ranged from 2.7 to 3.1%, fat content 17.4 to 37.9%, protein content 2.2 to 3.0% and total sugar 1.7 to 26.4%. Smoked banana/banana figs contained 337 kcal, 12.5% moisture, 0.2% fat, 5.1% protein content and 78.8% carbohydrates and contributed substantial amount of potassium (950mg/100g) and sodium (840mg/100g).

P86 - Studies on blending of banana and pine apple juices for making ready-to-serve beverage

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Commercial processing of bananas is limited to a few products; the major portion of total world production is consumed as fresh fruit. In some countries, banana juice is blended with orange juice and consumed as a breakfast drink. We studied the quality and acceptability of blends of banana and pineapple juices in different ratio and their dilutions for making ready-to-serve beverages. Banana and pineapple juices were blended in 90:10, 80:20 and 70:30 ratio and 30, 40 and 50% of blended juice were utilized. The results show that there was no significant variation in the total soluble solid and acid content of the different blends or dilutions. The total sugars were higher in beverages made with 40% pulp of 80:20 banana/pineapple blend. The clarity of beverage decreased with increase in pineapple juice level. The scores for colour and flavour increased with increase in the proportion of pineapple juice as well as increase in the level of juice blend. There was no significant variation in the scores for consistency and taste of different blends and dilutions. It was therefore, concluded that 70:30 banana and pineapple blend was best for making beverages having 40% juice, 15^{o} Brix total soluble solids and 0.30% acidity.

P87 - Banana research and development in the Federated States of Micronesia

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Banana and Plantain are the most important staple food and exported agriculture produce in the Federated States of Micronesia (FSM). Research and development activities on banana using biotechnology tools were initiated in Kosrae, FSM in 1998. A laboratory for micropropagation study, Micronesia Plant Propagation Research Center, (MPPRC), was established in 1999. Since then tissue culture procedures for ten locally grown bananas of economic or nutritional significance were established. Eight of the local banana varieties including two endangered Fei' banana varieties very high in β-carotenes (precursor of Vitamin A, a vitamin deficient in the FSM population) were micropropagated and distributed to farmers in all the 4 States in the FSM with the assistance from the Department of Interior, USA. An integrated conservation program for banana in the FSM is being established combining *in situ* and *in vitro* conservation methods. Micropropagation facility was extended to include a microbiology facility by 2001 and collaborative research aimed at banana improvement (developing disease resistance through *in vitro* breeding) is being undertaken in collaboration with the University of Guam, Guam, USA, with the assistance from USDA.

P88 - Improving food and livelihood security among smallholder banana and plantain producers in sub-Saharan Africa

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The push to increase banana and plantain production in sub-Saharan Africa has led to increased yield of these commodities in many countries in the sub-region. But turning the surplus from the increased yield into income and into development is a goal, which is still far from being achieved in many of these countries. The reason for this slow take-off is due to the fact that the use of modern facilities for post-harvest handling including packinghouse operation, CA storage, processing, among others, can not be justified as of now for handling produce from smallholder producers. Organizing smallholders into industrial organizations or getting them to enter into an industrial partnership with large-scale commercial ventures in the sub-region may be one of the possible avenues for solving the post-harvest and marketing problem. In this paper, possible approaches that could lead to viable post-harvest technologies as well as a sustainable improvement in food and livelihood security for rural smallholder banana and plantain producers are examined and discussed.

P89 - Research activities to promote intensive banana cultivation in subtropical Mauritius

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Banana, introduced in Mauritius in 1606, is one of the most locally appreciated fruit. However, until recently research and development was concentrated on sugar cane, the leading contributor to the GDP of the agricultural sector. Out of the 519 ha under banana cultivation in 2003 (predominantly 'Dwarf Cavendish'), over 95% were on slopes, growing under marginal and rainfed conditions. Rainfall is erratic and not uniformly distributed throughout the month, due to which the large water requirement of banana plants is not met. The already water-stressed banana plants further suffer from severe leaf tearing by cyclones and anticyclones, with a consequent poor yield (17-22 t/ha). On the other hand, due to the absence of market regulations for sale in local outlets growers are little concerned about improving yield and quality. However, with the uncertainty of the sugar market and the increasing number of supermarkets imposing quality criteria, the banana sector is attracting more investments and consequently research activities have been developed to enhance adoption of modern cultural practices.

Recent trials have shown that water, wind and temperature are the three main factors influencing local banana production. Under rainfed conditions, advantages normally associated with tissue culture material have not been obtained. Plant yield of 'Dwarf Cavendish' (tolerant to cyclones) was very low (22 kg bunch in the short dry season and 14 kg in the prolonged dry season), compared to drip-irrigated plots (30 kg bunch). The rainfed tissue culture plants also had a reduced rate of plant development, taking over 12-16 months to flower. To facilitate technology adoption on-farm, demonstrations of modern cultural practices are being set up on arable, fertile land and with drip-irrigated tissue culture plantlets of cv. 'Dwarf Cavendish'.

Mauritius is frequently visited by cyclones and affected banana trees may take over 1 year to return to production, as was the case after cyclone Dina in 2002. New growers have realized the importance of erecting windbreak (artificial or natural) to reduce wind damage. On-going trials clearly demonstrate that protected plants have a significantly larger bunch (28 kg compared to 21kg) due to a larger canopy.

Other on-going activities to sustain the rapid development in banana production in Mauritius include (i) variety evaluation (using FHIA hybrids, 'Williams', 'Dwarf Cavendish') under different agro-climatic conditions with aim of selecting high yielding, locally acceptable, disease tolerant and drought tolerant varieties (ii) *in situ* and *in vitro* multiplication of quality material, leaf disease control and (iii) guided tours/trainings to illustrate pre- and post –harvest handling practices.

P90 - Postharvest characteristics of three somaclones of FHIA-21 (AAAB)

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Taking into account postharvest characteristics is essential when evaluating somaclones of bananas. In the present study, we evaluated the behaviour of three somaclones IBP 14-23, IBP 17-13 and IBP 24-14) produced by the in vitro mutagenesis of the banana hybrid FHIA-21 (AAAB). The evaluated characteristics were bunch weight, the number of fingers and hands per bunch, the weight, length and diameter of the fruits, the weight of the pulp and of the peel, the total soluble solid content and pH. The results show that the somaclone IBP 14-23 produced the highest bunch weight, the highest number of fingers and hands and a pulp and rind not different from the control FHIA-21. The colour of the pulp of the somaclone IBP 17-13 was orange to dark orange. The total soluble solid content in the somaclones IBP 14-23 and IBP 17-13 was not significantly different from the one in the control. All the somaclones tended to be acidic. With respect to resistance to black leaf streak disease, IBP 14-23 IBP 17-13 displayed the lowest indices of infection and the highest number of functional leaves.

P91 - Development of fruit rolls from banana

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Fruit rolls, also known as fruit leather, can be manufactured by dehydrating fully ripe fruit puree. They are a tasty, thin, chewy, leathery dried fruit product and eaten as a confection. Tropical fruits such as banana, mangoes, guava, soursop and jackfruit are suitable for production of fruit rolls. This article focusses on the processing of fruit rolls from banana.

Several cultivars, especially those eaten raw were tested. The cultivars 'Berangan', 'Mas' and 'Embun', a Cavendish, were acceptable for fruit rolls. Cooking bananas can also produce good tasting fruit rolls. Among the varieties tried were 'Nangka' and 'Kapas'. 'Berangan' had a very good flavour and a nice yellowish colour. Other cultivars were whitish. 'Nangka' had a slight sour taste but was acceptable. 'Mas' turned darker faster than the other cultivars. Pre-treatments such as steaming or blanching before peeling were carried out to prevent browning during processing. The texture of fruit rolls may harden during storage. The use glucose syrup or honey and citric acid in the formulation reduces this problem. Pectin was added to give a leathery texture and to remove them easily from the drying trays. Other food conditioners such as maltodextrin were added to give a better texture.

Drying of fruit rolls can be done on non stick trays or normal trays but lined with plastic film before cooked fruit puree was poured on to the trays. The first method was preferred to produce wrinkle-free rolls. Drying was normally carried out using an electric dehydrator at 60^{o}C for 5-6 hours until dry and leathery so that the roll can be pulled out from the trays without breaking. The rolls had to be lined with a plastic film to prevent sticking before cutting and rolling. Sun drying is not recommended because the fruit puree will be spoilt. The moisture content of fruit rolls should be about 15-18 %. Storage can be done in laminated plastics or aluminium laminated foil for longer keeping.

P92 - Banana confectionery jellies

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Confectionery jellies made with ‘Mas’ and MB2 banana have been developed. ‘Mas’ was suitable as raw material for confectionery jelly processing as it gave a good flavor and orangey color. However, MB2 gave a whitish color and a plain flavor although acceptance increased when coloring was added. MB2 jellies with orange, apple green and egg yolk colors scored higher in color scores compared to plain MB2 jellies ($p=0.01$). In order to boost the acceptance, flavour could also be added.

The banana made up 17% of the product’s weight; the rest was sugar, glucose syrup, pectin, acid and sodium citrate. All of the ingredients, except acid, were cooked to 78 ºBrix and the pH, 3.3-3.6, was adjusted by controlling the addition of acid. The product was dried in starch mold for 6 hours, cleaned and sprinkled with sugar. The product was then ready for consumption.

‘MB2’ needs to be steamed prior to reducing it to prevent browning. However, steaming causes the water in the banana to ooze out, thus more banana is needed in order to get the required weight. Blending ‘Mas’ banana and water in the ratio 4:3 produced an excellent jelly product compared to 1:1, 3:2 and 7:5 ratios. In the case of ‘MB2’ banana, the amount of water used was 13 % or a 4:3 ratio of banana to water.

P93 - Effect of temperature on colour, chlorophyll content and chlorophyll bleaching activity during fruit ripening

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Changes in peel colour, chlorophyll content and chlorophyll oxidase activity were examined during the yellowing of bananas (Cavendish subgroup) ripened at 18±2 and 27±2°C. A temperature of 18±2°C hastened the ripening to a golden yellow within 5 days. In contrast, a temperature of 27±2°C did not induce the peel to turn to yellow even though the pulp had softened. Chlorophyll *a*, *b* and total chlorophyll content of the ripe bananas at both temperatures decreased significantly as ripening progressed. However, after ripening, chlorophyll *a*, *b* and total chlorophyll levels were higher in bananas ripened at 27±2°C than in those ripened at 18±2°C. Cavendish ripened at 18±2°C showed higher levels of chlorophyll oxidase activity after 1 day.

P94 - Banana powder and its premix production

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Banana is commonly used as an ingredient in the preparation of traditional cakes. Banana powder was prepared with the cultivar 'Mas' according to the developed processing procedure using drum dryer. The banana powder was used as substitute for fresh banana in the development of three traditional cakes in the premix form. The premixes were formulated based on the original formulation using fresh banana and they were processed by dry mixing the banana powder with wheat flour, followed by other ingredients such as sugar and others as listed in each product formulation. The mixtures were sieved, packed in paper/ aluminum/polyethylene and repacked in individual boxes and ready to be further processed into traditional cakes. The premixes can be kept for more than six month at ambient temperature.

Banana powder and its premix contained low moisture content and high carbohydrate content. Thus, banana powder can be used as a substitute for fresh banana in making banana-based product such as traditional cakes, cookies, snacks, chips, crackers, beverages, bay food and sauces. The acceptability studied showed that the traditional cakes produced by using flour premix and fresh banana were highly accepted by the taste panelists. This indicated that banana powder had no influence on the sensory attributes evaluated. Development of banana based traditional cakes would result in less time for traditional cakes preparation and more time for family members. There is also less waste disposal from food preparation.

P95 - Quality of frozen breaded banana

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Coating-freezing technology can be used to produce a new type of healthy, frozen banana-based product that is made with real banana fruit pieces. The product is a battered and breaded product produced in convenient form using either cooking banana or dessert banana varieties. In this study, an attempt was made to utilize cooking banana varieties for making frozen breaded product. 'Nangka', 'Gading' and 'Abu' were used. The products were packed either in high density polyethylene bags or aluminium laminated bags and repacked in a box individually as a secondary packaging material. The products need to be kept frozen until consumption.

The characteristics compared were percentage of edible portion, percentage of pick-up, recovery, chemical composition and sensory attributes such as flavour, colour, taste, texture and oiliness. Sensory evaluation results indicate that the fried coated banana of the three varieties studied were highly accepted by the taste panelists. The products resembled banana fritters. However, results indicated that 'Gading' was most suitable to be developed into breaded banana because it showed a high percentage recovery due to its thinner skin; it had the highest banana taste or flavors, attractive golden colour and lowest oil absorption upon frying.

P96 - Utilization of banana in jam product development

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Jam, jelly, preserves, conserves, marmalades and fruit butters are similar products. All are made from fruit, preserved by sugar and thickened or gelled to some extent. The principal objective pursued in the production of the jam is to provoke the same emotions as when eating fruit straight from the tree. Jams have conventionally been produced from temperate fruits such as apricot, blackberry, raspberry, strawberry, blueberry, plum and tropical fruits such as pineapples. In this study, an attempt was made to utilize bananas for making jams. ‘Mas’ and MB2 were used. The characteristics compared were texture, taste, aroma, flavour, sweetness, sourness and spreadability. Results indicated that ‘Mas’ was most suitable to be developed into jam due to its attractive golden colour, pleasant banana taste, flavour and aroma, good spreadability and cloudy jelly appearance. Sensory evaluation results indicated that ‘Mas’ banana jam was highly accepted, which means that it could be commercialized. If implemented, this would provide a solution to wastage problem in times of glut season.

P97 - Traditional ripening technologies in Nigeria

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The study examined the use of indigenous ripening technologies by banana and plantain women marketers in Enugu State, Nigeria. Data were collected from 40 banana and plantain women marketers using a semi-structured interview schedule. Frequency distribution, percentage and mean score were used in the analysis of the data. The findings revealed that the mean age of the banana and plantain women marketers was 31.5 years and majority (67%) of them were married with a mean family-size of 5. A greater percentage (52%) of the respondents had primary school leaving certificate of education, while about 35% of them had no formal education. The primary occupation of 78% of them was banana and plantain marketing, while their mean marketing experience was 7 years. Different indigenous ripening technologies were being used. The most common ones used containers such as drum, wooden boxes, pots and baskets in a cool dry place. The use of the indigenous ripening technologies helped the women generate a relatively constant income, which they used in purchasing more containers, more bunches of banana and plantain, essential household materials, provision of more food and balanced diet, good health care and possession of more livestock etc. The implications of the findings for banana and plantain indigenous ripening technologies extension education will be discussed.