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Mitigating the threat of banana *Fusarium* wilt: understanding the agroecological distribution of pathogenic forms and developing disease management strategies

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2 Executive summary

Fusarium wilt disease is a major banana production constraint in Indonesia, caused by the fungus *Fusarium oxysporum f.sp cubense* (*Foc*). A virulent strain named Tropical Race 4 (TR4) attacks many local banana varieties. Since the early 1990s, the disease has caused multi-million dollar losses. It is a serious threat to nearby Papua New Guinea where bananas are important source of foods.

This project aimed to reduce the spread and damage of *Foc* by understanding the distribution of its various races and developing mitigation measures. Project activities included: disease-mapping survey; participatorily identifying and evaluating appropriate disease management practices, and building capacity of farmers and of extension and quarantine officers. Coordinated by Bioversity International[1], this project involved national research and quarantine institutes in Indonesia and PNG, with the technical participation of the Department of Employment, Economic Development and Innovation[2] (DEEDI), Australia.

277 infected samples were collected from 16 provinces in Indonesia. 34 samples were collected in 7 provinces in PNG, focusing on provinces bordering Indonesia. Samples were sent to DEEDI for pathogen identification and Vegetative Compatibility Group (VCG) analyses. The project's findings highlight the widening distribution of TR4 *Foc* in Indonesia, and its spread and apparent predominance over other VCGs since previous surveys were conducted, which emphasises the importance of preventing its spread to other areas within Indonesia and neighbouring countries as infected planting material continues to be moved to new areas. Results showed that none of the samples from PNG was infected with Fusarium wilt, indicating that *Foc* is unlikely to have yet spread there. However, TR4 was already observed in the adjacent Indonesian province of Papua. In Indonesia, 8 VCGs were identified. VCG 1213/16, associated with TR4, was found in 62% of the samples, with highest incidence in Aceh and West Sumatera followed by VCG 01218 (12%) and VCG 0120 (10%) (VCGs associated to less virulent Race 1) (See tables 1 & 2 section 7), Many local cultivars were affected by Fusarium wilt, with Barangan (AAA), an important dessert cultivar being the most frequently sampled with VCG 1213/16 infection. The VCG analyses were corroborated by DNA analyses[3]. Samples were stored at DEEDI as part of a regional collection of *Foc* for future research. It was also found that Blood disease, a bacterial wilt disease is equally destructive as Fusarium wilt. Project surveys have provided a wealth of data on the current status of banana pests and diseases in the region ,and farmers will be more able to deal these pests and disease problems as a whole rather than individually..

Virulence tests suggested an interaction between VCGs and varieties, indicating differential susceptibility of various cultivars to various VCGs. This allows for targeted cultivar deployment and development of set of differential varieties as diagnostic tool to identify disease races. This paves the way for the next research step of validating this interaction in actual field conditions.

Results also indicated that some soil physical, biochemical, and biological properties may influence soil conduciveness/ suppressiveness to Fusarium disease. These observations

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[1] *The work was formerly coordinated by INIBAP (International Institute of Banana and Plantain), which became part of Bioversity International in 2006.*

[2] *formerly QDPI*

[3] *Amplified Fragment Length Polymorphism (AFLP)*

will lead to further research to understand and use soil the suppressiveness concept in improving disease management strategies as a part of the follow-up project (see below).

The survey also gathered information on farmers' perceptions of their production problems. Small farmers do not often implement any disease control tactics due to lack of resources, and technical know-how. Farmers also can't differentiate Fusarium wilt from blood disease. Each disease may require different control tactics. Another important result of the survey is the observed relative increase in incidence and spread of Banana Bunchy Top Disease. This further complicates the already disease-burdened banana production of small-scale banana farmers.

Some disease management practices were evaluated with farmers' participation in two locations. Results showed that improving production practices such as using clean planting materials, and population management through cultivation and plant spacing showed potential of not only reducing infection but increasing yield in both mixed and single cropping. There is however a need to sustain an affordable source of seedlings for farmers. Results suggest that *Foc* is best controlled by using an integrated production systems approach, taking into consideration existing cropping systems and other pest and disease constraints.

Training on disease diagnosis and management was given to farmers, and extension and quarantine officers in Indonesia and PNG, to increase awareness and capacity to prevent spread, to increase readiness, and to help mitigate the damage and threat of *Foc*. Throughout the conduct of this project participating national researchers improved their technical capacities through continuous interactions with research partners within the country and international partners.

The results of the project were reviewed by external reviewers together with national partners and ACIAR representatives before the project ended. They were also presented to the steering committee meeting of the Banana Asia Pacific Network (BAPNET). It was recommended that a new project phase be implemented, to upscale *Foc* management, together with blood disease in an integrated cropping system-community approach to create more impacts and sustainability. HORT 2008/040 titled, "*Integrated crop production of bananas to manage wilt diseases in Indonesia and Australia*" was thus developed and funded, and began implementation in July 2009.

3 Background

Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most devastating banana plant diseases, and is a major concern for banana producing countries. The damage potential of the disease is exemplified by the devastating outbreaks that occurred in Latin America in the 1950s, which destroyed whole plantations and led to the disappearance of the Gros Michel cultivar from the commercial dessert banana industry (Simmonds, 1966). *Foc* is conventionally classified into four pathogenic forms known as “races”. Race 1, which destroyed the Gros Michel plantations, also attacks many local cultivars in Asia; Race 2 affects specific cooking bananas; and the particularly virulent ‘Tropical’ Race 4, affects a wide range of cultivars including Cavendish, and cultivars susceptible to Race 1 and 2. TR4, the extremely virulent strain of *Foc* , has caused substantial production losses for commercial and subsistence farmers in Indonesia, Taiwan, Malaysia and the Northern Territory of Australia. *Foc* is also classified into vegetative compatibility groups[4] (VCGs). Twenty-one clonal lines, or VCGs, of *Foc* are known to exist. Recently, severe infections were reported in Cavendish plantations in China and the Philippines. The variation in pathogenicity within the conventional ‘races’ highlights the need for more precise characterization of variability based VCGs and/or molecular techniques, and a better understanding of the relationship between pathogenicity and *Musa* diversity.

Foc cannot be effectively controlled with fungicides and spores remains over a long period in the soil. The early and accurate diagnosis of the disease, prevention of its spread and the deployment of management strategies are, therefore, of utmost importance. The impact of the disease has prompted the Banana Asia Pacific Network (BAPNET) to call for increased research support into pathogenic variability, host-resistance and sustainable disease management methods to alleviate the losses caused by this disease.

The project proposed to carry out a comprehensive survey and characterization of *Foc* pathogenic forms and to develop national strategies for disease exclusion, containment and management, identification of a package of management tools through participatory approaches, and exploration of existing networks to enable a “fast-tracked” adoption of effective measures. The project complemented the ACIAR bilateral project CP 2004/034, "*Diagnosis and management of wilt diseases of banana in Indonesia*". Specific areas of synergy and complementarities between projects are elaborated in the next section. The main outputs of the project include a characterized collection of *Foc* strains, distribution maps of *Foc* pathogenic forms, validated diagnostic tools, a manual of farmer-evaluated tactics for disease management, national *Foc* management strategies and improved capacity for disease exclusion, containment and control. The expected impact of the project is to reduce losses and the risk of losses to Fusarium wilt of banana in smallholder and commercial production in Indonesia and PNG. Furthermore, through the successful execution and the scaling up of the project’s outputs, the risk of the spread of the disease to countries within the region and beyond will be reduced.

This project focused its activities in Indonesia and Papua New Guinea (PNG) with the participation of advanced research laboratories in Australia. Indonesia was one of the first countries to be affected by Tropical Race 4 and the development of the banana export industry has been severely affected by the disease. Fusarium wilt Race 1 has already been reported in PNG near the border in Irian Jaya. The virulent Tropical Race 4, although still absent, poses a serious threat to the unique cultivars grown by the large majority of smallholders.

4 based on the ability of the mycelium to anastomose to form heterokaryon to determine genetic relatedness (Puhalla, 1985

The project was coordinated by the Bioversity International's regional office in Asia-Pacific located in the Philippines, and implemented through national partners in Indonesia and PNG, who played major roles in conducting in-country surveys, developing participatory disease management strategies and national disease control strategies. The department of Economic, Employment Development Innovation (DEEDI, formerly QDPI&F) provided expertise in physiological and genetic analyses, diagnostic tools and techniques. BAPNET countries and members of ProMusa, the global programme of more than 100 Musa researchers and in particular its working group dedicated to Fusarium wilt, were consulted to share their experiences and expertise in managing the disease.

Partner country and Australian research and development issues and priorities

During the BAPNET Steering Committee meeting in Indonesia in 2003, the directors of the Indonesian Centre for Horticulture Research and Development (ICHORD) and Indonesian Tropical Fruit Research Institute (ITFRI) identified Fusarium wilt as one of the major constraints to banana production in Indonesia and stressed the need to develop a system for sustainable disease management (Dimyati, 2004). In the province of Lampung alone, Fusarium wilt caused farmers US $9.1–10.6 million in yield losses between 1993 and 2002 (Nurhadi et al, 1994). Around 5000 ha of export Cavendish bananas established in the early 1990s by Del Monte and Chiquita were totally abandoned because of Tropical Race 4. Furthermore, three locally important commercial cultivars – Barangan, Raja Serai and Ambon – are all susceptible to the disease. The concerns over the impact of the disease led to the development of an ACIAR bilateral project CP 2004/034 (*Diagnosis and management of wilt diseases of banana in Indonesia*). The project complements the initial project, and shares expertise, experience and information on the disease, as well as cultural and other production practices such as the low cost delivery system for disease-free planting materials with BAPNET member countries such as Taiwan, the Philippines and Australia.

Fusarium wilt Race 1 has been reported in PNG specifically in Bengawi, West Sepik province along the border with Papua, Indonesia (formerly Irian Jaya) (Shivas and Philemon, 1996). Although Tropical Race 4 has not yet been reported in PNG, VCG 1213/16, regarded as the dreaded Tropical Race 4, has already been reported in nearby Papua, attacking Pisang Rajah (AAB) near Timika in 1997, at Merauke in 1998, and attacking Pisang Barangan (AAA) in Biak in 1997 (Davis et al, 2000). Merauke is just 70 km to the border of PNG. The movement of Tropical Race 4 may take the same route as Race 1, and certainly poses a serious threat to small-scale growers in PNG. The indigenous cultivars grown by subsistence farmers in PNG have tested susceptible to the disease, so its spread represents both a threat to food security and to the country's unique banana biodiversity. An early detection system and regular monitoring along the border is essential to prevent the disease's incursion and minimise potentially disastrous losses. An evaluation of local cultivars in PNG for their susceptibility to different pathogenic forms of *Foc* will provide a basis for determining appropriate responses in the event of a disease incursion.

Tropical Race 4 has also appeared in the Northern Territory, Australia where quarantine measures, the use of disease-free planting materials and good farm practice, have so far served to prevent the spread of the disease to other major banana-producing areas. Diagnostic tools for rapid identification of the disease and specific pathotypes have also been developed.

A survey on the geographic distribution and variability of *Foc* was conducted in Indonesia and Malaysia in 1997 by INIBAP and partners in Asia, including QDPI&F, with funding from ACIAR. Further advances have led to the characterization of *Foc* isolates based on VCGs, DNA Amplification Fingerprinting (DAF) and various molecular markers (Bentley, et al, 1998).

In the past 10 years, significant progress has also been made by researchers in various countries towards identifying options for managing the disease; for the most part these are yet to be validated and adopted by smallholder farmers. Options included the introduction of disease-resistant varieties available through the INIBAP genebank, the use of disease-free tissue-cultured planting material, selection of resistant clones (somaclonal variants) through participatory approaches, and disease management approaches based on biological control and crop husbandry (Hwang, 2004). Given the level of information and the tools and technologies available, especially through peer-networking as provided by Bioversity and ProMusa, countries are in a good position to deal effectively with the disease through national level strategic planning, detailed diagnosis and customised disease containment and management measures. The project used these elements to address the disease where it occurs at its most diverse and in its severest form in Indonesia, as well as in PNG, where good planning has the potential to avert any serious incursion of the disease.

The project aligns with expressed priorities in ACIAR's Annual Operational Plan concerning the maintenance and enhancement of smallholder incomes and sustainable management, particularly of soils, in PNG and the management of major pests and diseases of banana in Indonesia.

Research and/or development strategy and relationship to other ACIAR investments and other donor activities

Australia, through ACIAR, was a co-founder of International Network for Improvement of Banana and Plantain or INIBAP, (now Commodities for Livelihoods Programme of Bioversity), and has continued to support its work. Australia is also a founder member of BAPNET, which was initiated in 1991 as a result of a task force funded by ACIAR and AIDAB (Australian International Development Assistance Bureau). Between 1987 and 1989, DPI&F collaborated with IPGRI to collect *Musa* germplasm in Papua New Guinea. ACIAR funded the publication of this work in a *Musa*logue, a catalogue of *Musa* diversity, devoted to Papua New Guinea. DPI&F also co-sponsored a subsequent Musalogue, which covered the diversity in the entire genus *Musa*. Diversity of *Musa* species provides an important gene-pool for sources of resistance to banana diseases.

Earlier ACIAR projects such as CS1/1990/033 *Banana improvement in the Pacific Islands*, CS2/1993/726 INIBAP's Asia Pacific Network (ASPNET) and CS1/1995/707 - *Evaluation of Sigatoka and Fusarium wilt resistant hybrids and cultivars in Tonga and Queensland* looked at banana improvement, screening species and cultivars, and surveying pest and diseases and their management. Two of these projects involved participation by INIBAP. A survey on the geographic distribution and variability of *Foc* was conducted in Indonesia and Malaysia in 1997 by INIBAP and partners in Asia, including the then DPI&F, with funding from ACIAR. Also funding from ACIAR has led to further advances in the characterization of *Foc* isolates based on VCGs, DNA Amplification Fingerprinting (DAF) and various molecular markers. In particular, a PCR (Polymerase chain reaction)-based diagnostic test has been developed for the detection of Tropical Race 4.

DPI&F continues to be involved in INIBAP's International *Musa* Testing Programme (IMTP), under which DPI&F is actively studying Fusarium wilt. DPI&F also collaborates with INIBAP to screen Fusarium populations and study pathogen diversity. Some work has been carried out on the less virulent Fusarium strains affecting banana, their impacts on Australian banana production, and their control with *Mycorrhiza* and *Pseudomonas*. Australian Commonwealth Scientific and Research Organization (CSIRO) have also carried out limited work on alternative hosts for *Foc* Race 4.

The CP2005/136 project brought together the collective expertise and most advanced technologies developed in the past 10 years. The approach used was to work with national agricultural systems in Indonesia and PNG and with banana farmers/growers on the ground in Indonesia, to build capacity and customize available technologies for use at

local and national levels. The emphasis is on building coordinated national strategies for an already-affected country (Indonesia) and an urgently threatened country (PNG) that might be used as models for other countries in the region, while also bringing a range of measures directly into the field to allow the fast adoption of those that have proved effective.

The project built a firm foundation of information and risk assessment, based on mapping the incidence of disease, including the various pathogenic forms of *Foc* (Races), and the distribution of affected/unaffected *Musa* cultivars. The expertise and experiences of the Pro*Musa* Fusarium working group and of other experts from a range of countries who have been actively addressing the disease, such as China, Taiwan as well as Australia, will continue to be shared through workshops or consultations to assist in strategy building organized by BAPNET.

The approach in PNG focused on surveys, assessment of risk and the development of a national strategy for the deployment of preventative measures and the planning of a quick response if a *Foc* outbreak should occur. One practical tool that was identified in the course of the project is the possibility of developing a set of differential varieties that respond differentially to various *Foc* pathotypes and allow the rapid and simple identification of the pathogen in the farmers' fields. National repositories for banana germplasm in the partner countries were involved in the establishment and maintenance of such cultivar collection. Capacity-building to enhance preparedness was the key element of the project.

In Indonesia, a range of management measures: integrating resistant varieties, clean planting material, biocontrol methods and crop husbandry, were evaluated in farmers fields and customized to local needs and conditions, allowing the rapid adoption of those measures that were found to be acceptable and effective. The experiences of the farmers and their use of the different options was analysed. In these activities, there was close coordination with the ACIAR bilateral project CP 2004/034 to ensure that each project carries out their work plan in synergy with the other and learns beneficially from the other. To aid this process several Indonesian staff worked on both projects and joint planning and review meetings were held. Specific areas of synergy and complementarities between both projects are as follows:

1. To collect and characterize Foc and expand the reference collection with representative isolates:

CP 2004/034 focuses on the survey of existing management knowledge in targeted areas, and the diagnosis of two types of banana wilt: Fusarium wilt and bacterial wilt. Management knowledge known in the surveys identified with farmers the "best bet" practices validated and promoted. The geographic focus of the survey is West Sumatra, Central Java, North Sumatra, East Java, East Kalimantan, South Sulawesi, and Lampung. Samples of diseased plants were collected in target areas and diagnosed at a species level only.

CP2005/13: covers a broader geographic area, including Indonesia and PNG. While CP2004/034 focused on the diagnosis of banana wilt pathogens, CP 2005/136 characterized *Foc* at the VCG and Race level. Geographic areas not surveyed by CP2004/034 were included in the survey of CP2005/136 covering West Java, Aceh, Irian Jaya, North Sulawesi, Southeast Sulawesi, and Nusa Tenggara Barat. Surveys were also carried out in PNG (Sandaun, Western Bougainville and Manus). Duplicate samples taken by CP2004/034 were provided to partners in CP2005/136 for analysis to VCG level, which helped provide a more complete picture of *Foc* distribution in Indonesia and PNG. Both projects provided samples to the reference collection at DPI&F.

The survey instrument initially drafted for use in CP2004/034 was discussed and revised at a joint workshop in February 2006 involving scientists from both projects. The agreed

survey was used by both projects so that comparability of results would be ensured. A joint survey instrument was used to monitor uptake of technology and impact. This project addressed quarantine as an important element of disease containment with implications at a global level.

2. To establish pathogenic relationships between VCGs and various banana cultivars:

CP 2004/034 focused on PCR-based diagnostics specifically for 'tropical' race 4.

CP 2005/136 focused on evaluating and validating diagnostic tools (including Amplified Fragment Length Polymorphism AFLP) for *Foc* VCGs. One of the objectives of this project was to understand the pathogenicity / virulence of VCGs to different cultivars, hence developing a "set of differential cultivars" that may serve as a diagnostic tool and guiding framework for disease management.

3. To develop and validate disease management tactics:

CP 2004/034: focused on reviewing existing management tactics in Indonesia through the survey. The following tactics were evaluated: use of hygiene, biocontrol agents, soil amendments and varieties.

CP 2005/136 draws from experiences in and outside Indonesia; "best bet" technologies used in BAPNET member countries were reviewed and evaluated through participatory approaches in demonstration plots. Disease management approaches for evaluation included the following: modified annual cropping systems using disease-free planting materials used in Taiwan; clean seed systems similar to those used in the Philippines; eradication and treatment methods employed by private companies; and integrated use of resistant varieties from the INIBAP national repository centres. Soil health indicators relating to vulnerability to *Foc* was also developed and validated based on the results of the Banana Root and Soil Health (BRASH) project of Australian partners.

Manuals and other technical materials developed by CP2005/136 were provided to CP2004/034 for dissemination, promotion and adoption by banana growers through the provincial extension partners of CP2004/034.

4. Capacity enhancement and training:

This activity focused on capacity building in soil health diagnostics and a production systems approach to disease management for banana growers (smallholder) extension workers; and PNG quarantine officers in diagnostics for early detection, interception and incursion management.

All strategies and documented practices were published and disseminated as Global Public Goods through Bioversity and BAPNET's communication programmes, and the experiences of Indonesia and PNG conveyed to other countries in the region through BAPNET and Bioversity coordinated meetings.

4 Objectives

Objective 1. : To collect and characterize *Foc* (with special reference to Tropical Race 4) in Indonesia and PNG, and expand the regional reference collection with representative isolates.

1.1 Activity : Survey, collect, conserve and characterize *Foc* samples in Indonesia, and PNG

1.2 Activity : Enhance and validate molecular characterisation tools (DNA Amplification Fingerprinting (DAF) and AFLP).

Objective 2. : To elucidate the pathogenic relationships between VCGs and various banana cultivars

2.1 Activity : Conduct pathogenicity tests of VCG group isolates against a range of *Musa* cultivars and varieties, to elucidate the relationship between VCG and pathogenicity.

Objective 3. : To develop and validate disease management tactics appropriate to the severity of local disease forms, varieties grown and local practices

3.1 Activity : Review disease management options, including those proposed or validated in previous or existing projects (ACIAR and BAPNET projects) such as the modified annual cropping systems using disease-free planting materials used in Taiwan, clean seed systems similar to those used in the Philippines; eradication and treatment methods employed by private companies; integrated use of resistant varieties from the INIBAP national repository centres and disease management approaches based on biological control, elicitors and crop husbandry.

3.2 Activity : Evaluate disease management options formulated in Activity 3.1 in demonstration-trial plots in Indonesia through farmers-participatory methodologies.

3.3 Activity : Assess and validate diagnostics tools for soil health and other parameters appropriate for use in developing disease management strategies, as well as in understanding the pathogenic variability and dynamics of the pathogen.

3.4 Activity : Analyze, document and promote successful disease management options and their applicability to different production systems.

Objective 4. : To build the capacity of national researchers and quarantine personnel in Indonesia and PNG to design and implement disease prevention and disease management measures for use against *Foc*

4.1 Activity : Training of researchers, extension workers, quarantine personnel in methodologies for disease management and prevention.

4.2 Activity : Carry out risk assessment to identify districts according to the risk and impact of *Foc* infection and contribute to national strategies for disease exclusion, containment and management.

5 Methodology

Objective 1: To collect and characterize Foc (with special reference to Tropical Race 4) in Indonesia, and PNG, and expand the regional reference collection with representative isolates.

Survey and collection of samples

Personnel from ITFRI and NARI were given training at a Bioversity International - coordinated workshop in Malaysia in April 2006 on Fusarium wilt diagnosis, sample collection and processing, as well as a basic introduction to VCG analyses and characterization by experts based at QDPI&F and the Forestry and Agricultural Biotechnology Institute (FABI), South Africa.

The surveys were carried out by national partners. Survey and collections were carried out within the frameworks of the 2 ACIAR projects, CP 2004/034 and CP 2005/136. CP2004/034 focused on areas where the disease outbreaks were reported to be severe while CP 2005/136 concentrated on zones where the crop has socio-economic importance and those regions that are excluded from the former (i.e. West Java, Aceh, Irian Jaya, North Sulawesi, Southeast Sulawesi and Nusa Tenggara Barat). The scope of the survey aimed to provide as complete a representation of *Foc* distribution as possible, including areas where the disease has not been reported. In PNG, the survey focused in provinces bordering Indonesia such as Sandaun, Western Bougainville and Manus.

Three survey teams of three personnel, including one local agricultural officer, worked in parallel, working in adjacent geographical areas to make optimum use of resources. A common survey instrument has been developed for use in both projects CP 2004/34 and CP 2005/136. Survey sites were mapped using global positioning system (GPS) technology

Aside from collecting disease samples, associated information were collected. These included the identity of *Musa* cultivars (affected and unaffected), disease symptoms, agro-ecological and climatic parameters, type of production system, farmers' broad socio-economic profile, perceptions of disease and management practices. One important parameter that was determined was about soil-health indicators. These were assessed using a soil-health kit developed at DEEDI. ITFRI researchers were trained by experts from DEEDI before the conduct of the survey. Bio-chemo-physical properties were collected in areas where the diseased samples were collected.

To add value to the survey, ITFRI, NARI and DEEDI banana taxonomists joined the survey teams to identify and collect new banana accessions that maybe resistant or used in resistance breeding against Foc as part of the overall Foc mitigation system. Collected varieties were deposited at the Musa genebanks of ITFRI in Indonesia and NARI, in PNG.

Characterization of samples

Collected Fusarium samples for both CP 2004/034 and CP 2005/136 were maintained at ITFRI in Indonesia and at NARI in PNG. Duplicates were sent to QDPI&F for characterization and conservation as an international reference collection. VCG was determined using the technique described by Puhalla in 1985. In this technique, isolates were assigned to VCGs based on heterokaryon formation between complementary nitrate-nonutilizing (nit) mutants produced on media supplemented with chlorate. Nit-mutants were produced for all isolates as well as for the known VCG tester strains. The nit-1 and nit-3 mutants were then paired with each of the nit-M tester strains on minimal medium (MM) as described by Correll and his group in 1987. Nit-M, nit-3 and nit-1 mutants of the same isolate were also paired to test for self-compatibility. Complementary nit-mutants that formed dense, wild-type growth on MM were assigned to the same VCG.

Vegetatively incompatible isolates were detected by their inability to form a heterokaryon when paired on MM. All samples were characterized through VCG analyses. A representative subset was also characterized using the DNA analyses depending on VCG, source and host variety. Molecular characterisation was performed using DNA Amplification Fingerprinting (DAF) and complemented with a newer method, Amplified Fragment Length Polymorphism (AFLPs).to characterise *Foc* isolates. AFLPs had been used to characterise race 1 and 4, and 16 VCGs of *Foc* (Groenewald et al, 2006). The method for characterization was refined and validated for use by QDPI&F staff. Refinement and validation included testing a wider range of isolates (including race 2 and all VCGs currently represented), optimisation and standardisation of protocols.

Objective 2: To elucidate the pathogenic relationships between VCGs and various banana cultivars.

Based on the results of the VCG analyses from DEEDI, the duplicate samples at ITFRI representing the VCGs identified in Objective 1, were reproduced for inoculum production used to challenge selected varieties representing various genomic groups. This was done to establish the relationships of VCGs and different varieties in terms of virulence and pathogenicity The first trial was done in the screenhouse. Seven VCG groups: 01213/16, 0123, 0120, 0124/5, 01218, 0126 and 01219 were used to challenge 25 varieties. Two-month old in-vitro meriplants were carefully removed from the media to avoid roots damage and washed with running tap water. Those with healthy white roots were selected for inoculation by dipping the roots of the meriplants in 10^6 conidia suspension for 5 minutes (Figure 1). Inoculated plantlets were planted in 220 ml plastic cups containing sterile sand and watered with liquid compound fertilizer (Hyponex). Each cup (with a plantlet) was then placed into another empty plastic cup, so called double cup method, to collect the excess water and thus prevent the spread of *Foc* infected soil from the inoculated.

The following data were taken:

(1) Incubation period: Observation was taken from the first external symptom, yellowing on the edge of leaf lamina. Incubation period is calculated from the time of inoculation to the first appearance of external symptom.

(2) Disease severity data was based on -- Disease severity on the leaf using the Leaf Symptom Index (LSI) and Rhizome Discoloration Index (RDI).

(3) Disease incidence was calculated as percentage of plants showing Foc symptoms against the total experimental plants in a treatment

Figure 1. Dipping of banana meriplant roots in 10^6 conidian suspension for 5 minutes

Field validation of virulence studies

The field verification study was limited to using only one VCG1213/16 to avoid introduction of VCGs in the field. VCG1213/16 was used as it was the most virulent pathogen and was found to occur in the experimental field at ITFRI. The field trial was laid out in randomized complete block design, 2 blocks. Twenty five accessions were tested on each block and each accession contained 12 plants. Planting materials consisted of tissue-culture derived meriplants planted in polyethylene bags and maintained in the screen house for two months. Prior to planting, two-month old in vitro plants were inoculated with *Foc* suspension (10^6 spore/ml) of VCG 01213/16 (TR4) by pouring 5 ml of this suspension onto the plant medium. The plants in the field were given the standard cultural practices. Symptom appearances were monitored to calculate incidence, incubation period and rate of disease development.

Objective 3: To develop and validate disease management tactics in Indonesia appropriate to the severity of local disease forms, varieties grown and local cropping practices.

Details of disease management strategies implemented in BAPNET member countries were collated by the BAPNET secretariat. These included the deployment of improved varieties that have been evaluated against Tropical Race 4 in Malaysia, Indonesia and Taiwan as part of the IMTP; experiences of Taiwan and Australia in developing model systems for quarantine; use of clean planting materials; and improved production practices such as population management. The use of disease-free planting materials using tissue-culture was the major foundation of the IPM-cropping system approach that was introduced. The availability and affordability of the tissue cultured materials to small-scale growers was the key to the success of such an approach. This project adapted the Philippine experience on developing an effective delivery system of clean planting materials to small-scale farmers. A survey of private tissue culture laboratories in Indonesia was carried out to develop private-public partnerships in making clean seedlings available and affordable for small scale farmers. Commercial laboratories now supplying the needs of large plantations in Indonesia were identified and tapped to produce inexpensive rooted-tissue culture to be sold to small-scale nurseries in different banana growing provinces. A delivery system was developed and nursery managements were taught to enterprising small-scale nursery owners who would in turn supply the needs of small-scale growers in the community.

Existing practices employed by Indonesian farmers, identified through survey, and those developed through the activities of CP 2004/034 on biocontrol agents, and resistant varieties were also included in the collation. The range of management tactics was characterized and evaluated in an inception workshop involving national stakeholders from Indonesia and key researchers from Australia, Indonesia, and INIBAP. The workshop reviewed the applicability, cost-effectiveness, flexibility and potential effectiveness of management strategies in the context of the prevailing conditions and practices in Indonesia. Management options were chosen for evaluation in two project sites in demo-trial pilot plots. Partners at ITFRI established the demo-trial plots in farmers' field. Researchers, extension workers and farmers in the project sites were trained to implement, adapt and evaluate the selected methodologies.. Due to some delays of field establishments, the previously scheduled organized field days were not organized. However, during the conduct of the field demo trials extension workers and farmers were invited for field visits that served as a learning situation for all.

Objective 4: To build the capacity of national researchers and quarantine personnel in Indonesia and PNG to design and implement disease prevention and disease management measures for use against *Foc*.

Personnel in partner institutes in Indonesia and PNG were trained in the following methodologies to support the implementation of Objectives 1-3: protocols of field surveys of disease incidence and processing of samples; household socio-demographic survey techniques; rapid participatory appraisal techniques, protocols for pathogenicity/virulence tests and soil health studies; implementation and evaluation of specific disease management tactics; and other diagnostic tools for disease management and quarantine. Training materials in the form of manuals were produced.

The participatory appraisal meetings with farmers related to the demo-trials were used as an avenue of training for farmers and extension workers who were participating in the project. In the conduct of the field trials, farmers in the community were invited to visit the demo plots as an avenue for capacity building.

Towards the end of the project, training programs on diagnosis of banana diseases with emphasis on banana wilts were conducted in PNG and Indonesia. These were participated in by extension and quarantine officers. In PNG it aimed to provide extension and quarantine officers the needed knowledge and skills to identify possible incursion of the disease thus allowing them to isolate and eradicate to prevent establishment and apread. Lectures and field activities on early disease diagnosis and mitigation measures were included.

To expand the dissemination of research outputs on the VCG characterization and geographic mapping of Race distribution of occurrence, data were consolidated and presented in national meetings and workshops.

6 Achievements against activities and outputs/milestones

Objective 1: To collect and characterize* Foc *(with special reference to Tropical Race 4) in Indonesia, and PNG, and expand the regional reference collection with representative isolates.

no.	activity	outputs/ milestones	completion date	comments
1.1	Collect, conserve and characterize *Foc* samples in Indonesia and PNG	National/ regional maps of the geographic distribution of *Foc* races/ VCGs and cultivars affected - Indonesia	May 2009	Surveys for both the multilateral and bilateral projects were completed in 16 major banana producing regions/ provinces of Indonesia: (1)Aceh, (2)North Sumatra, (3)West Sumatra, (4)Lampung, (5)West Java, (6)Yogyakarta, (7)East Java, (8)West Kalimantan, (9)Central Kalimantan, (10)South Kalimantan, (11)East Kalimantan, (12)South Sulawesi, (13)North Sulawesi, (14)Southeast Sulawesi, (15)Papua and (16) West Nusantara A total of 277 samples were collected; 178 samples were sent to QDPI&F for VCG analysis; the rest remained at ITFRI since the Australian import permit of sampling materials expired toward the third year of the project (DEEDI was following up the renewal of the permit).The remaining samples are stored properly in ITFRI. Eight *Foc* VCGs were isolated during the survey, namely: 01213/16; 0123; 0124/5; 01218; 0120; 0126; 01219; 0121; 1213/16, the identifying VCG for the virulent TR4, was frequently found in almost all provinces with highest incidence in Aceh and West Sumatera. While TR4 was observed in the province of Papua PNG, the extent is less than in other Indonesian islands. This however poses threat to PNG where *Foc* has not been yet been identified. 62% of the samples were of VCG 1213/16 followed by VCG 01218 (12%) and VCG 0120 (10%) (VCGs associated to Race 1). Many local cultivars were affected by Fusarium wilt, with Barangan (AAA), an important dessert cultivar being the most frequently sampled with VCG 1213/16 infection

				The survey of the different banana producing provinces led to the identification of several common banana pests and diseases in Indonesia namely: (1) Blood disease; (2) Fusarium Wilt; (3) Bacterial corm rot; (4) Sigatoka; (5) Banana skipper; (6) *Cordana musae*; (7) Banana bunchy top; (8) Thrips; (9) Weevils; (10) Borers The survey also yielded good information on farmers' perception of the problem and coping mechanisms they employ in addressing these problems. 58% farmer-respondents complained against pests as the major constraint for banana production. The two most important are Fusarium wilt and Blood Disease. Farmers rarely distinguish the two wilt diseases. Farmers rarely employ any disease management measures against diseases. If any, they just simply abandon their crop and change it to other crop species. Due to farmers' practice on distribution, dispersion of planting materials; banana bunchy top virus (BBTV), and weevil borer are considered as new potential threats after blood disease and Fusarium wilt. Actual results of the survey indicated that BBTV is spreading rapidly in many provinces in Indonesia, making it a potential serious problem like Foc and Blood Disease.
		National/ regional maps of the geographic distribution of *Foc* races/ VCGs and cultivars affected - Papua New Guinea	May 2009	Surveys in 7 sites in PNG have been completed namely: (1) Western Province; (2) Sandaun Province; (3) Manus Province; (4) North Solomon Province; (5) East New Britain Province; (6) Eastern Highlands Province; (7) Morobe Province (Markham Valley). A total of 34 samples were collected from banana plants showing Fusarium wilt - like symptoms. The pseudo stem samples were sent to QDPI for analysis. No *Foc pathogen* was recovered from any of these samples. Common diseases observed in the area are *Marasmiellus* rot caused by *Marasmiellus inoderma*, Sigatoka, black cross, cordana leaf spot and leaf speckle.
			May 2009	A follow-up survey was conducted in May 2009 in Western Province based on previous reports of some incidence on bananas in remote areas of the province. The following sites were visited: Hopnai Village, Matkomnai Village, Ningerum Station, Kiunga Town, Gwari CIS station, Samogos DPI station and South and Middle Fly Districts. No plants showing symptoms of Fusarium wilt disease were observed in the areas surveyed.
			March 2010	In all provinces (except East New Britain), interviews with farmers were conducted. A total of 54 farmers were interviewed. From the survey the following information were gathered: (1) Bananas are usually planted in home gardens less than 0.5ha, with less than 50 plants per garden. (2) Men mostly prepare the land and women do the planting, harvesting and marketing of the fruits (3) At harvest, 50% of the fruits are used for home consumption and the other half usually sold in the local market (4) From bananas, the farmers earn approximately K10/week (USD3.50), less than 25% of family income. (5) majority of farmers use bananas as fresh food (raw or cooked). Commonly grown varieties were: Kalapua No.2; Daru; Kekiau; Tukuru No.2 (6) The diseases that affect production are black sigatoka, black cross, Cordana and leaf speckle, Marasmiellus rot and scab moth
			March 2010	Twenty nine new banana accessions were collected during the survey in Manus Province, Sandaun Province and Western Province. The accessions collected were added to the existing germplasm collection of NARI in Laloki. The new accessions

				are currently being characterized and documented. Considerable diversity of bananas was observed in Manus province. In summary, 22 AA, 6 AAA, 10 AAB, 9 ABB, 1 AB, 2 ABBT, 1 AAT and 2 Fe'i types of bananas were found in the area. During the survey, the team were able to collect 10 native banana varieties not yet present in the banana germplasm collection at NARI in Laloki
		Variety Evaluation	March 2010	10 cooking -type varieties and 5 dessert-type varieties were selected from the PNG National Banana Germplasm Collection (NBGC) based on their popularity. The varieties are currently in the field for multiplication and later will be used to establish agronomic evaluation trial. The trial will be conducted 2nd quarter of 2010.
1.2	Enhance and validate molecular characterization tools	Conservation of *Foc* samples	May 2009	All samples received have been placed in long term storage at QDPI, Brisbane. As the isolates were characterised they were transferred to long term storage by lyophilisation (freeze-drying). Pieces of agar plus carnation leaf are placed into vials under sterile conditions before being lyophilised in a Heto LyoLab 3000 freeze dryer. All 111 Indonesian isolates received as part of Hort/2005/136 and 67 samples received from Hort/2004/034 have now been freeze dried. Duplicate vials of each isolate are being held in cold storage under quarantine conditions at the Indooroopilly site.
		Refined molecular tools for identification and characterization of VCGs/ races	May 2009	156 out of 178 samples had DNA extracted and subsequently tested for TR4 using PCR. TR4 test was found to cross amplify non-TR4 isolates. TR4 PCR may need to be redesigned. 75/118 were characterised using AFLPs with 4 primer combinations. AFLPs generally clustered VCGs together, therefore can be considered a suitable method for characterising isolates.

PC = partner country, A = Australia

Objective 2: To establish pathogenic relationships between VCGs and various banana cultivars.

no.	activity	outputs/ milestones	completion date	comments
2.1	Conduct pathogenicity tests of VCG isolates against a range of *Musa* cultivars and varieties. To elucidate the relationship between VCG/ Race and pathogenicity	Diagnostic tools in the form of a 'differential' set of varieties with varying susceptibility to *Foc* VCGs	March 2010	Pathogenicity tests of VCG isolates from Indonesia against a range of *Musa* cultivars was conducted both in screenhouse and on the field. Sreenhouse test: 14 accessions were tested namely: Barangan, Ketan, Karuk, Randah, Calcuta, Mas, Kilita, Perancis, Ambon hijau , Ambon Kuning , Kepok kuning , Raja bulu, Raja sere , Rejang. These cultivars were inoculated with the following VCGs: 01213/16; 0123; 0124/5; 01218; 0120; 0126; 01219
				In the screenhouse test, inoculation was conducted using tissue cultured banana meriplants. The roots were dipped in 10^6 spore/ml conidia suspension for 5 minutes. The inoculated plantlets were replanted in 220ml cups containing sterile sand. Seedlings were watered with liquid compound fertilizer (Hyponex). Each seedling cup is placed in another cup to collect excess water.
				The following data were taken: (1) Incubation period: Observation was taken from the first external symptom, yellowing on the edge of leaf lamina. Incubation period is calculated from the time of inoculation to the first appearance of external symptom. (2) Disease severity data was based on -- Disease severity on the leaf using the Leaf Symptom Index (LSI) and Rhizome Discoloration Index (RDI). (3) Disease incidence was calculated as percentage of plants showing Foc symptoms against the total experimental plants in a treatment
				Based on the results of screenhouse data, VCG 1213/16 (TR4) was the most virulent among the 8 VCGs causing infection on all varieties tested. Eight test varieties were found highly susceptible (HS) to VCG 01213/16 namely Barangan (AAA), Ambon Hijau (AAA), Ambon Kuning (AAA), kepok kuning, Raja Bulu (AAB), Mas (AA), Kilita (AAB), Calcuta 4 (AA) and Randah (AAA). Raja Sere (AAB) and Rejang (AA) were found relatively less susceptible to *Foc* TR4 VCG. One accession, Perancis (ABB), was found resistant (R) to VCG 01213/16 The accession Perancis was also found resistant to VCG 0123, 01218 and 0120. The accessions Pisang Mas, Ambon Kuning,and kilita were found highly susceptible to all VCGs tested. Kepok Kuning(ABB) showed symptoms in all the VCGs tested, an unexpected results for a Balbisiana. Although it showed wilting and yellowing the degree of wilting were not severe as the other varieties, but by virtue of incidence (%) this variety was classified as highly susceptible. The accession Pisang rejang showed a mechanism of recovery when challenged to VCG 0124/5. The tested plants initially died, a new healthy sucker emerged at 30 days after inoculation. The sucker grew well was categorized as tolerant to the VCG.

no.	activity	outputs/ milestones	completion date	comments
				Field validation of virulence studies: The field verification study was limited to using only one VCG1213/16 to avoid introduction of VCGs in the field. VCG1213/16 was used as it was the most virulent pathogen and was found to occur in the experimental field at ITFRI. The field trial was laid out in randomized complete block design, 2 blocks with 12 plants per accession. To ensure uniform inocula, 2-month old tissue culture-derived seedlings were root-inoculated by pouring 5ml of 10^6 spore/ml conidia suspension of VCG 01213/16 per seedling before planting. Field disease assessment data confirmed that Barangan was highly susceptible to TR4. Ambon Hijao, Ambon Kuning, Raja Bulu Mas, Kilita and Berlin showed intermidiate to susceptible disease reaction. Calcutta 4, Parancis, Kepok Kuning showed high field resistance as there were no plants showing any external symptoms. Some inconsistencies of screen house and field results maybe improved by improving disease severity assessment methods and standardizing methods of inoculation and incubation of inoculated plants. Although VCG1213/16 proved to be the most virulent, the results of the screen house and field verification tests clearly indicated that there is an interaction between VCG and varieties. This suggests that varieties can be used as differential set for diagnostic purposes, and that cultivar deployment maybe used as a tool for managing *Foc*. The recovery mechanism of Pisang Rejang also suggests that there may exist various resistant mechanisms especially under field conditions. It is very important to pursue this concept of VCG by Variety interaction to clarify the concept of Races and their use in disease management.

PC = partner country, A = Australia

Objective 3: To develop and validate disease management tactics in Indonesia appropriate to the severity of local disease forms, varieties grown and local cropping practices.

no.	activity	outputs/ milestones	completion date	comments
3.1	Participatory approach of determining "best bet" disease management options, based on regional, national and local experiences, that will be evaluated/validated in farmers demo-trials.	A set of "best bet" options/tactics to manage the Fusarium disease, that could be validated/evaluated in Indonesia; including a low cost delivery system of clean seedlings for small scale farmers.	June 2007	From the participatory workshop with farmer-co-operators in June 2007, the following were the identified 'best-bet' options for Fusarium wilt management in the demonstration plots: (1) the use of disease-free planting materials obtained from tissue culture propagation; (2) the use of tolerant/resistant varieties to Fusarium wilt such as FHIA-17 (AAAA), FHIA-21 (AAAB), FHIA 25 (AAAA), GCTCV 119 (AAA), Ketan-01 (AAB) and one susceptible variety Ambon Warangan (Cavendish subgroup, AAA); (3) treatment by rice-hull burning on infected mats for spot eradication; (4) the use of good cultivation practices such as the application of balance fertilizer, good planting distance, number of plants per mat, field sanitation and the use of cover crop, and (5) the use of a bio-control using Pseudomonas fluorescence adopted from the farmers the field-school program of the Assessment Institute for Agricultural Technology.

no.	activity	outputs/ milestones	completion date	comments
3.2	Evaluate management options formulated in Activity 3.1 in demonstration- trial plots in Malang and Lampung, Central Java, Indonesia through farmer-participatory methodologies	Best bet options validated under farmers field, and documented	November 2007	The establishment of field trials for the best-bet options started with a participatory workshop/meeting with farmers of Dampit (Srimulyo) and Lampung, extension workers (BPTP) and ITFRI researchers conducted in November 2007 to discuss the best bet options and management practices to be included in the demo farms. Farmers were given options to choose options or combinations of options at their discretion according to their interest and capabilities. The best bet options suggested in both demo sites were the following: (1) Varieties: FHIA-17, FHIA-21, Ambon Kuning; (2) Planting materials: tissue culture plants; (3) Good culture practices: single crop/mix cropping systems; population management; organic & inorganic fertilizer, bagging, de-suckering, etc; (4) Infected plant management: detection, eradication and heat treatment These workshops with farmers, extension workers and researchers also served as capacity building in introducing new elements of interventions such as the use of clean planting materials. Farmers and extension workers were trained in handling and nursery management of banana tissue cultured seedlings and the cultural practices needed in growing them in the field. Other management options were alo introduced such as new varieties, diagnosis, eradication and treatment, biological control. In both locations most of the farmers used the mix-cropping system, using the popular varieties Ambon Warangan, Kepok Kuning. Some farmers included FHIA 17 and FHIA 21. Farmers who participated in using the some project interventions used tissue culture seedlings, planted in straight row planting, as a single crop or in mix-cropping with Nilam, coffee, cassava, ground nut and ginger.. Some farmers did not any adopt any intervention but just using their traditional practice. One of the problems encountered in the field trial is the delay in the delivery of planting materials thus delaying the execution of the project. As of this writing of the report, the field demos are still going on. Those farmers which planted susceptible varieties suffered high infection of Foc. FHIA 17 and 21 were significantly less affected by *Foc*, and yielded bigger bunch and fruits. However, the fruits did not command good price as these are not yet readily accepted by the market. In both cases, straight row planting and population management through de-suckering showed healthier and bigger plants and fruits. Preliminary results indicate that farmers prefer mix-cropping bananas with high value crops such as Nilam, ground nut and ginger.

no.	activity	outputs/ milestones	completion date	comments
			May 2009	In Lampung, banana plants were mixed planted with groundnut and ginger. By the end of 2009, Fusarium wilt in Lampung demoplot almost wiped out Ambon Kuning variety (67.33%), but not the introduced varieties FHIA 17 and 21. Aside from Fusarium wilt, Ambon Kuning was also affected by Sigatoka leaf spot and stem borer. Another disease that threatens the orchard was BBTV that initially appeared on FHIA 17. Both FHIA 17 and 21 performed good agronomically in Lampung, with an average bunch weight of 22.4 to 28.7 kg. Learning from the situation, the farmer co-operators in the area intended to replace Ambon Kuning with the introduced varieties. However, the problem was the marketing of the new varieties, because the varieties were newly introduced, market responded poorly to the varieties and commanded low price (Rp. 600 – 800 = USD 60 – 800 cents per kg). The introduced varieties would be given more value if processed as banana chips. The farmer co-operator requested a bunch of banana fruits of FHIA for processing.
3.3	Develop and evaluate a delivery system of affordable clean planting materials to small scale growers through private company-public partnerships	A delivery system of affordable clean planting materials available to village levels as part of the IPM system, evaluated, documented and established	May 2009	Private companies producing tissue cultured meriplants for banana were identified and listed (Appendix 11) Further activities of this objective will be continued in the new ACIAR project (HORT 2008/040).
3.4	Assess and validate diagnostics tools for soil health and other parameters appropriate for use in developing disease management strategies in Malang and Lampung	Soil health or other indicators suitable for use in disease management and soil health discussion paper	August 2008	Along with the surveys in different provinces of Indonesia, the following soil measurements were taken from each survey location: Soil textural class Soil temperature Electrical conductivity pH Nitrate nitrogen Disease data Cultivar data

no.	activity	outputs/ milestones	completion date	comments
				Most complete data sets were those from the provinces of West Java, Lampung, West Sumatra, Aceh and West Kalimantan. A multivariate analysis of *Foc* on bananas determined from a survey in Indonesia revealed that where bananas were grown on acid soils with high nitrate-N levels in the soil, the incidence of *Foc* was likely to be higher. Soils with a neutral soil pH and low EC and nitrate-N content tended to have a lower incidence of *Foc*, even if the disease is present. Furthermore, in very acid soils the incidence of *Foc* is low if EC and nitrate N are low. Analysis of single parameters, such as soil texture, pH, EC and nitrate-N, failed to adequately explain the variation in the incidence of *Foc*. Even when the same banana cultivar was investigated on a similar soil type, such as Barangan grown on clay soils, the incidence of Fusarium wilt was poorly explained by the EC of the soil. To adequately understand the role soil health and different soil properties have on the incidence of *Foc* of banana further studies are required in more controlled conditions which measure a greater number of soil parameters that account for physical, chemical and biological soil properties.
3.5	Analyze, documents and promote successful management options and their applicability to different production systems	Summary and analysis of management options and their applicability and related public awareness materials	March 2010	During the duration of the project two introduced banana varieties FHIA 17 and 21 gave good agronomic performance and good pathological traits that can be used as part of the management options for banana cultivation. However, market acceptability of these varieties is poor; hence, an approach is needed in marketing and utilizing the varieties for processing. There were some inconsistencies observed on the response of some varieties towards Fusarium wilt pathogenicity in the screenhouse and field test. An improvement of the methodology and experimental set up is necessary to clarify the result. Improvements such as, identification of a new set of varieties that will be evaluated against Fusarium wilt in the demonstration plots based on the pathogenicity tests conducted in the project; field application of new set of management options; better identification of cooperating farmers and better site selection. This can be conducted in the next phase of the project (Hort 2008/040).

PC = partner country, A = Australia

Objective 4: To build the capacity of national researchers and quarantine personnel in Indonesia and PNG to design and implement disease prevention and disease management measures for use against Foc*.*

no.	activity	outputs/ milestones	completion date	comments
4.1	Training of researchers, extension workers, quarantine personnel in methodologies for disease management and prevention	Researchers and quarantine personnel trained	November 2007	Training on soil health indicators and survey methods was held at ITFRI on 6 – 8 November 2006, participated by 42 ITFRI researchers and technicians. Training *foc*used on the theories of soil health indicators and practical application and operation of the soil indicator kits, and survey methodology. After this 3-day training, participants were (1) able to understand soil properties related to plant health, (2) know what is needed to measure soil properties and (3)more skilled in using the soil test kit. Three soil test kits, containing 14 items, were prepared by Tony Pattison. The training was conducted in the classroom and field discussion and demonstration. Participants also experienced measuring the soil properties listed in the survey form under field condition within 15 to 25 minutes. Discussion on the survey methodology mainly *foc*used on how to select survey location, collect secondary data, and how to select farmers/ respondents.
4.1	Training of researchers, extension workers, quarantine personnel in methodologies for disease management and prevention	Researchers and quarantine personnel trained	October 2009	Training for quarantine officers and extension workers towards the end of the project was conducted at ICHORD Jl. Raya Ragunan 19 Jakarta on October 30 – 31, 2009. A total of 15 participants from - AIAT (Assessment Institute for Agricultural Technology), Quarantine personnel, university researchers, ITFRI, and ICHORD attended the training. The workshop discussed the next steps to mitigate the treat of *Foc* in Indonesia Training was conducted in Both Indonesia and PNG In PNG, the training was held in Lae. Extension workers and quarantine officers attended the training for the purpose of increasing PNG's capacity to for early detection, interception and incursion management of Fusarium wilt and other important banana diseases. Participants of the training were from the following institutions: (1) National Agriculture al Research Institute (NARI), (2) National Agriculture Quarantine Inspection Authority (NAQIA) and (3) Department of Agriculture and Livestock. The *foc*us of the training was to improve capacity of extension officers to manage incursions of banana diseases into PNG with emphasis on measures to prevent the spread of Fusarium Wilt across PNG Indonesia Boarder In Indonesia, training was conducted twice, at the beginning of the project (as part of preparation of project implementation) and at the end of the project to deliver information and technologies resulting from the project.

7 Key results and discussion

7.1 Survey, Collection and Characterization

7.1.1 Indonesia

Banana Fusarium wilt disease field surveys were conducted[5] in Indonesia in sixteen (16) major banana producing provinces namely: Aceh, North Sumatra, West Sumatra, Lampung, West Java, Yogyakarta, East Java, West Kalimantan, Central Kalimantan, South Kalimantan, East Kalimantan, South Sulawesi, North Sulawesi, Southeast Sulawesi, Papua and West Nusa Tenggara (Figure 2). A total of 277 samples were collected in both surveys. One-hundred-seventy-eight samples were sent to QDPI&F for VCG and DNA characterization, while the rest were analysed at ITFRI.

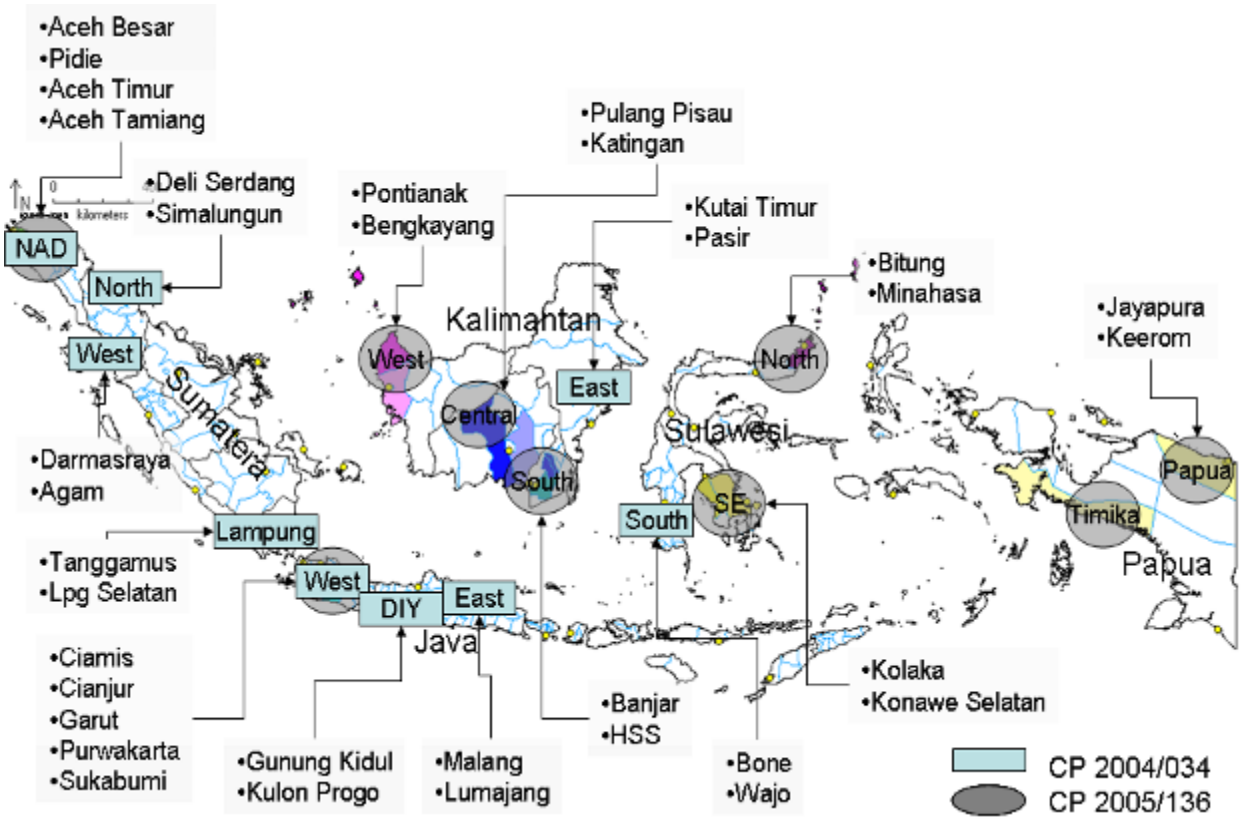

Figure 2. Districts where banana surveys were conducted in 2006 - 2009

Occurrences of the disease and their VCGs are shown through their geographic distribution (Table 1 and Appendix 1) and by the varieties they affected (Table 2). VCG 01213/16, the VCG associated to *Foc* Tropical race 4 (TR4) was widely distributed in the provinces of Aceh, West Sumatra, Yogyakarta, West Kalimantan, East Kalimantan, South East Sulawesi and North Sulawesi. This VCG affected all varieties but Barangan, Raja Bulu, and Ambon Putih were mostly affected (Appendix 2).

The data from the surveys conducted provided the information for the development of the distribution map. The map contains the information where the different VCG groups are located in Indonesia. When the characterizations of the pending samples are completed,

[5] *for both HORT 2004/034 and HORT 2005/136*

this can be a more comprehensive basis for quarantine measures and disease management system in the different provinces (Appendix 3).

Of the 178 isolates received by QDPI&F, 135 were assigned a VCG. The group of isolates which did not give a VCG result fall into several categories. Some cultures were irreversibly mutated before the VCG process began, so were not suitable for testing, and one isolate was determined not to be *Fusarium oxysporum*. Isolates which had typical, healthy *Fusarium oxysporum* morphology but did not yield a VCG result could possibly represent undescribed VCGs. Utilising DNA amplification fingerprinting, Bentley et al. (1998) identified Indonesian *Foc* genotypes which did not match existing VCGs. Another possibility is that isolates were not *Foc*. Several of the isolates which did not give a VCG result were from Pisang Kepok (Manurung/Saba - ABB) which is extremely susceptible to blood disease, caused by the blood disease bacterium. Blood disease has some similar internal and external symptoms to Panama disease and it is possible that non-pathogenic *F. oxysporum* may have been isolated from blood disease affected plants and assumed to be the pathogen.

Isolates collected during the project have tested positive for the following VCGs or complexes: 0120/15, 0121, 0123, 0124/5, 0126, 01213/16, 01218 and 01219. Tables 1 and 2 summarise the Provinces and varieties from which these VCGs were recovered. Positive recordings for *Foc* were made for all of the sampled provinces with the exception of West Nusa Tenggara; the single isolate collected there has not returned a VCG result. A summary of the positive samples sorted by province and host is presented in Tables 1 and 2, respectively. All of the strains identified have previously been detected in Indonesia, and most results obtained in this project (in terms of strain diversity, distribution and host varieties) are consistent with those of previous workers who have studied *Foc* in Indonesia (e.g. Pegg et al., 1994; Bentley et al., 1998; Buddenhagen, 2009). However, our knowledge of *Foc* diversity in poorly studied provinces, such as Aceh and those in Kalimantan, has been expanded as a result of this work. Previously, limited information on *Foc* diversity and distribution has been available for many Indonesian provinces.

VCG 01213/16 (TR4) is by far the most prevalent strain detected, accounting for approximately 60% (80/135) of the positive results. It was most commonly isolated from the varieties Barangan (Lakatan - AAA) and Pisang Raja (AAB). In this project, TR4 was detected in Sumatra (Aceh & West Sumatra), Java (West Java and Yogyakarta), Kalimantan (West and East Kalimantan), Sulawesi (South East and North Sulawesi) and Papua. It has also been reported from other Indonesian islands such as Bali and Halmahera (QPIF database, unpublished; Ploetz and Pegg, 2000) which were not surveyed during this project. Large export Cavendish plantations established in Indonesia in the 1990s were abandoned due to the disease (Nasir et al., 1999, 2003), and locally important commercial cultivars, such as Barangan, are especially vulnerable (Buddenhagen, 2009).

These findings highlight the wide distribution of TR4 *Foc* in Indonesia, and its spread and apparent predominance over other VCGs since previous surveys were conducted, which emphasises the importance of managing its spread to other areas within Indonesia and neighbouring countries. Infected planting material continues to be moved to new areas (within and between provinces), and TR4 is now found very close to the border of Papua New Guinea (PNG) in Papua Province (Davis et al., 2000), posing an imminent threat to the extensive banana diversity of that country.

Four isolates of VCG 0121 were detected in West Sumatra, all from the cultivar Buai, a Cavendish type (AAA). This strain has been shown to be closely related to VCG 01213/16 by a range of molecular techniques (Bentley at al., 1998; Groenewald et al., 2006), yet its distribution in Indonesia appears to be restricted to Sumatra. The factors limiting its spread are unclear.

Samples from diseased wild bananas *Musa schizocarpa* were collected from two locations between Jayapura and the PNG border in Papua Province. Isolates recovered from these plants were characterised as VCG 0126. Ploetz and Pegg (1997) suggest that the susceptibility of this *Musa* species to Panama disease is indicative of an historical absence of the pathogen from the island of New Guinea, where it originated. How these particular *M. schizocarpa* plants became exposed to the pathogen in an uncultivated forest verge situation is unknown.

A small number of VCG results which seemed anomalous, for example VCG 01218 in Ambon Hijau (AAA Cavendish type), was reconfirmed by repeated VCG testing and/or molecular analysis. These apparent anomalies can potentially be explained by misidentification of the banana variety in the field. Accurate identification may not always be possible, for example, because of the poor state of a plant due to disease or environmental factors. Alternatively, if the variety is correctly identified then resistance to non-race 4 strains may have broken down due to environmental stresses.

Relevant information was also identified from the results on socio-cultural-demographic surveys of the banana farmers. A total of 583 farmer respondents were interviewed. It was found that banana wilts are the main causes of low banana productivity, and that farmers often do not distinguish the difference between Panama Wilt and Blood Disease. Most of the respondents do not apply any control measures as they are not familiar with any measure because they seldom are in contact with extension workers. More than 90% of the banana farmers are men; the farmers were found to have an average of 7yrs education, less than 10% of the respondents had formal training on banana production. For banana production and management, none of the farmers uses tissue-cultured planting materials and chemical treatments. Most of the planting materials used in the areas were derived from suckers from their own plants and also from neighboring fields. For Aceh, the major banana disease identified was Fusarium wilt (76%).

Table 1. VCGs recovered from Indonesian provinces

VCG	Aceh	N. Sumatra	w. Sumatra	Lampung	West Java	Yogyakarta	East Java	West Kalimantan	Central Kalimantan	East Kalimantan	South Kalimantan	South Sulawesi	SE Sulawesi	North Sulawesi	Papua
01213/16	✓	✓	✓	✓		✓	✓	✓		✓			✓	✓	
01218	✓		✓		✓	✓		✓							
0123					✓				✓						
0120				✓	✓	✓	✓		✓						
0124/5					✓										
0126									✓		✓		✓		✓
01219						✓								✓	
01213	✓														
0121			✓												

Table 2. VCGs recovered from Indonesian banana varieties

VCG	*M. schizocarpa*	Barangan	Raja sere	Raja bulu	Ambon hijau	Ambon kuning	Siem	Pulo (ABB)	Raja	Nangka	Kepok	Candi	Rojomolo	Jantan	Panjang	Mysore
01213/16		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	
01218			✓		✓		✓			✓	✓					✓
0123			✓			✓	✓									
0120/15			✓			✓	✓		✓		✓					
0124/5								✓	✓							
0126	✓					✓	✓			✓	✓					
01219			✓		✓						✓					
0121					✓											

Weeds, pests and diseases were the identified major constraints for banana production in the areas surveyed. Based on the survey, 35% of the farmers identified diseases as the major constraint for production (Figure 3). Across the five main islands of Indonesia (Sumatra, Java, Kalimantan, Sulawesi and Papua), based on the survey conducted, Fusarium wilt and blood disease were identified as the major diseases for banana. Other leaf diseases such as Sigatoka leaf spot, Cordana leaf spot and black cross disease were commonly found in Papua. Banana bunchy top disease was found in Sumatra and Java and rarely found in Kalimantan, Sulawesi and Papua. Among the insect pests, stem borers were found common in the island of Sumatra, Java and Sulawesi. Other banana related insect pests were rarely found in the islands surveyed (Figure 4 and Appendix 5). A total of 48 varieties of bananas were observed during the survey in the islands of Indonesia. Five wild *Musa* varieties were also observed and collected. These samples are currently being conserved in ITFRI *Musa* germplasm collection (Appendix 6).

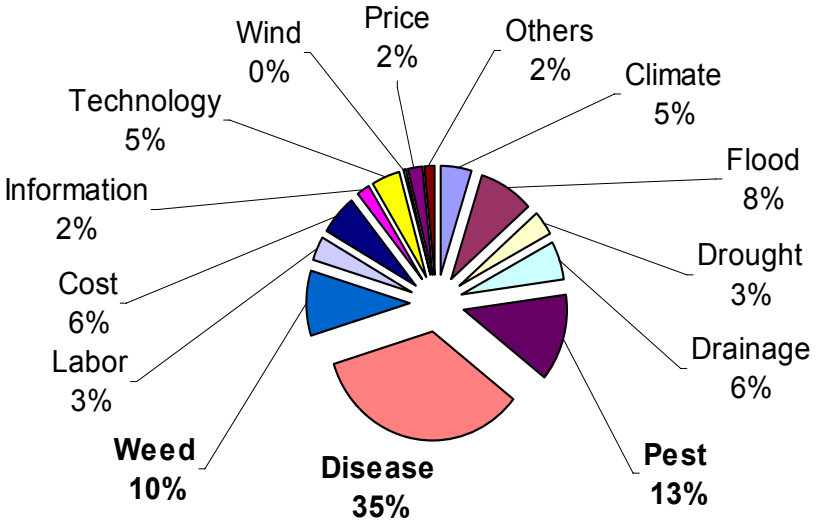

Figure 3. Major constraints for banana production based farmers' perception

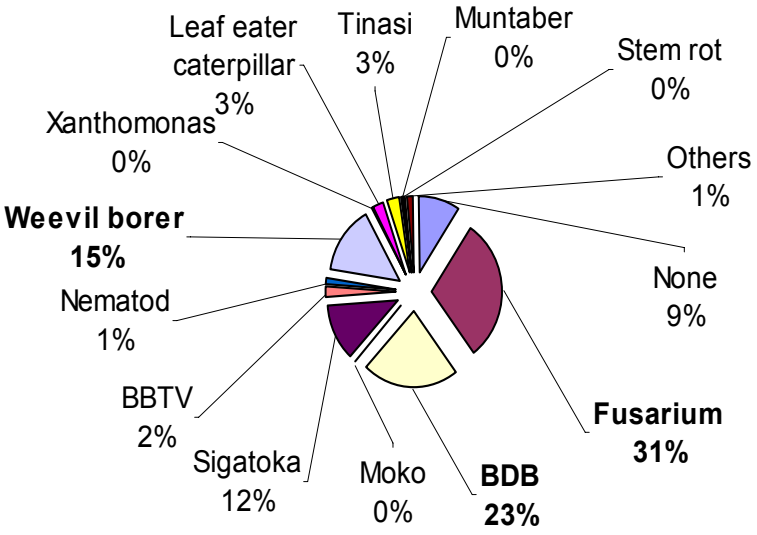

Figure 4. Major pests and diseases of banana production in Indonesia

A survey of bananas in different provinces of Indonesia took place in 2006 and 2007 to collect and categorise *Foc* and measure some basic soil properties. As the primary aim of the survey was to determine *Foc* severity and collect specimens of *Foc,* the selected indicators of soil properties needed to be measurable in the field, rapid to conduct and only require simple portable equipment. For this reason the soil indicators chosen were soil textural class, soil temperature, pH, EC and nitrate-nitrogen.

Only 38 sites were included in the analysis due to limited soil samples, relating the incidence and type of *Foc* VCG present to soil conditions. Across sites there was a range in soil properties, but most soils tended to have neutral pH (x = 6.9 (4.9-8.8)), low EC (x = 38 (4-96) µS m-1) and low nitrate-N (x = 7 (3.75-12.5) mg kg-1). The incidence of *Foc* showing symptoms averaged 24.7% of plants infected at a site, but ranged from (0.8-82.0 %).

Not surprisingly, no single soil indicator was able to explain the incidence of Fusarium wilt. Similarly, there were no trends of the incidence of *Foc* across different soil textural classes with soil properties. Most *Foc* samples isolated tended to be the VCG 1213/16 (67%). Furthermore, half of the samples that were positive for 1213/16 were isolated from clay soils and 60% of the of the samples positive for 1213/16 were from the cultivar Barangan. The high incidence of 1213/16 found on Barangan on clay soils allowed a correlation analysis, which was positive for the incidence of *Foc* and EC, explaining 61% of the variation in the incidence of *Foc* (*Foc* = 0.6965 x EC -15.2 R2 = 0.61 P<0.01). This relationship for Barangan on clay soils was simplistic and would need validation, as there are many other soil factors that need to be considered in the incidence of *Foc*. However, it does indicate that soil conditions may play an important role in the incidence of disease on bananas. The higher EC, where salinity is not an issue, generally indicates greater mineral salts in the soil which may be indicate nutrient availability. If this were true then Barangan cultivated on clay soils with high nutrient availability are more prone to a higher incidence of *Foc*.

A multivariate analysis of the data from 38 sites that had complete soil and VCG analysis of *Foc* allows a more holistic perspective of the survey information. A cluster analysis was able to form four groups from the data of soil properties and the incidence of *Foc* with a similarity to each other greater than 0.9; at this distance of similarity the samples evaluated can be divided into four different groups (Figure 5).

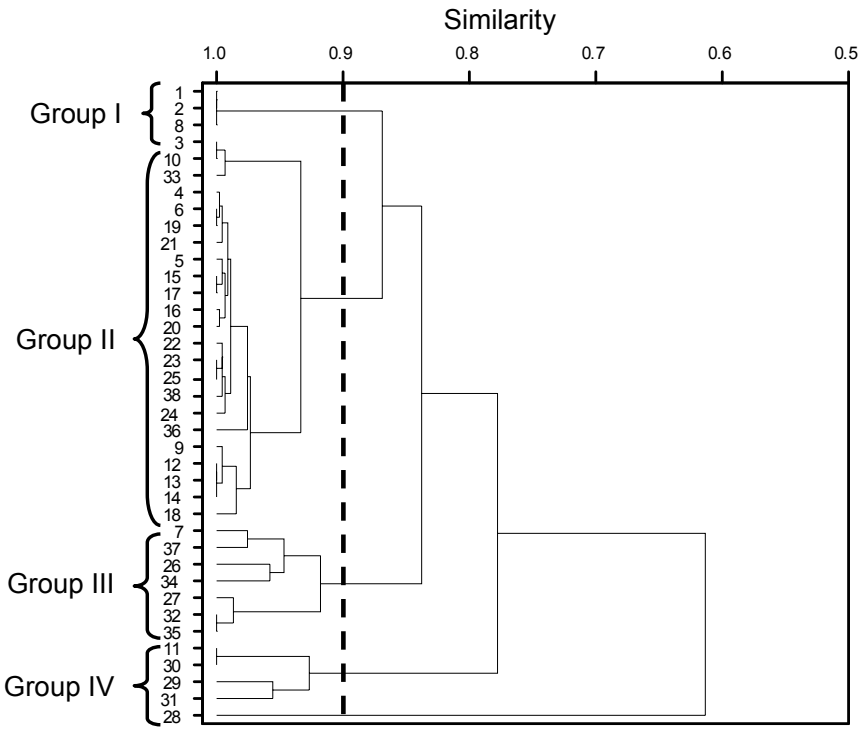

Figure 5. Cluster analysis grouping of 38 sites sampled for the incidence of *Foc* and soil properties in Indonesia. Numbers represent the location number and dashed line represents a similarity of 0.9.

One site, 28, had a similarity less than 0.6, but was placed in group IV having a similarity closer to this group than any other rather than forming a group which consisted of a single sampling site.

A principle component analysis of the sites using pH, EC, nitrate-N and incidence of *Foc* was able to explain 80.1 % of the difference in the four groups of farms (Figure 6). The first principle component was able to explain 46.4% and the second principle component able to explain 33.7 % of the variation between sampling sites.

Sites belonging to group I (3) tended to have low EC, pH, nitrate-N and incidence of *Foc* (Figure 6; Table 3). The sites belonging to group II, which was the majority of sampling sites (23), tended to have a neutral soil pH, low EC and nitrate-N and a lower incidence of *Foc* (Figure 5; Table 3). The sites belonging to group III (7) had a neutral to slightly alkaline soil pH, higher EC and nitrate-N and higher incidence of *Foc* (Figure 5; Table 3). The sites belonging to group IV tended to be more acid soils, with a high nitrate-N and moderate soil EC, but had a very high incidence of *Foc* (Figure 5; Table 3).

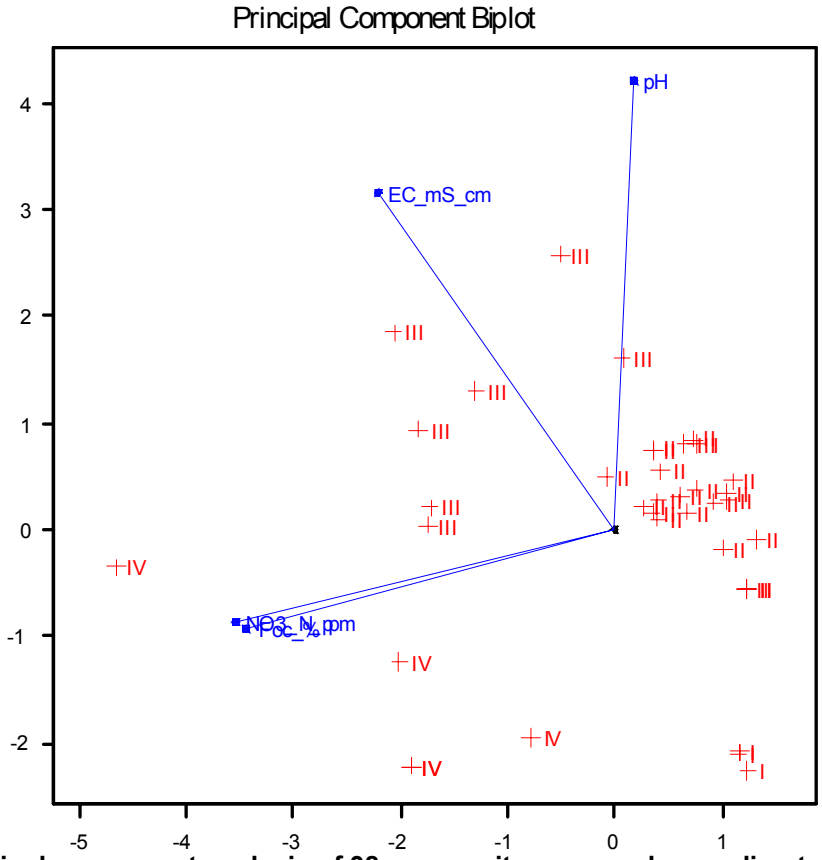

Figure 6. Principal component analysis of 38 survey sites grouped according to a cluster analysis of soil pH, EC, nitrate-N and incidence of *Foc* and the vectors showing separation of principal components.

Table 3: Means of *Foc* incidence, EC, pH and nitrate-N of 38 sites grouped according to a cluster analysis.

Group	*Foc* incidence (%)	EC (µS/cm)	pH	NO3-N (mg kg-1)
I	1.7	21	4.4	5.0
II	7.3	39	7.0	5.3
III	33.8	80	7.3	8.6
IV	61.8	39	5.6	14.0

The results suggested that the higher incidence of *Foc* in group IV sites tended to occur when acid soils had high nitrate-N content. Acid soils with low nitrate-N, that is group I sites, did not have the same incidence of *Foc*. Similarly, sites with a neutral soil pH, low nitrate-N and EC, that is group II sites, tended to have a lower incidence of *Foc*. Therefore, if *Foc* is present in the soil, then maintaining a neutral soil pH, and ensuring that there is not an oversupply of soil nutrients, particularly nitrate, allows the incidence of *Foc* to be slowed, compared to adding high amounts of nutrients and allowing the soil to become acid. However, this observation would need further validation. As nitrogen is an important nutrient in the growth of bananas a balance would need to be made between application of fertiliser to boost production and a reduction in fertiliser to slow the progress of *Foc*.

Principal component analysis of the 38 sites was performed using banana cultivars, provinces and *Foc* VCGs. However, these failed to adequately separate sites using the four parameters pH, EC, nitrate-N and incidence of *Foc*.

A multivariate analysis of *Foc* on bananas determined from a survey in Indonesia revealed that where bananas were grown on acid soils with high nitrate-N levels in the soil, the incidence of *Foc* was likely to be higher. Soils with a neutral soil pH and low EC and nitrate-N content tended to have a lower incidence of *Foc*, even if the disease is present. Furthermore, in very acid soils the incidence of *Foc* is low if EC and nitrate N are low. Analysis of single parameters, such as soil texture, pH, EC and nitrate-N, failed to adequately explain the variation in the incidence of *Foc*. Even when the same banana cultivar was investigated on a similar soil type, such as Barangan grown on clay soils, the incidence of Fusarium wilt was poorly explained by the EC of the soil.

7.1.2 Papua New Guinea (PNG)

There have been records from PNG of bananas infected with 'Race 1' *Foc* in a few isolated locations (Table 4) (Davis 2000; Davis et al. 2001). Although PNG is still reportedly free of the TR4 strain, it has been detected at a number of locations in the Indonesian province of Papua close to the PNG border. Therefore, there is an imminent threat of an incursion of this strain into the country with potentially devastating impacts on PNG banana production and that is why PNG was included in this multi-lateral project.

Surveys in PNG, were carried out in seven provinces: (1) Morobe Province/ Markham Valley, (2) Sandaun Province, (3) Manus Province, (4) North Solomon Province, (5) East New Britain Province, (6) Eastern Highlands and (7) Western Province (Figure 7). The survey was conducted in these areas because -- the identified provinces borders to Indonesia where TR4 has been identified, previous records of Fusarium Wilt exist for locations within those areas, or uncontrolled border exchange of planting materials occurs. For example Manus Province was chosen specifically because it is frequently visited by foreign fishing and logging vessels from SE Asia and Sandaun Province borders with Papua (Indonesia) where TR4 has been previously reported in this province. Surveys have also been completed in sites previously identified with the presence of Fusarium Wilt (race 1) in PNG namely, Lido, Bewani, Kiunga, Niogamban, and Kainantu.

Table 4. Fusarium oxysporum f.sp. cubense records in PNG (Davis et al. 2001) (DPI database, Linda Smith)

Date	Location	Cultivar/genotype	VCG	'Race'	Collector
1996	Bewani, Sandaun	Unknown (ABB)	0126	1	R. Shivas, E. Philemon
1998	Road to Bewani, Nth Vanimo, Sandaun	Unknown (ABB)	0126	1	R. Davis
1998	Kiunga, Western Prov.	Unknown (ABB)	0126	1	R. Davis
	Kianunta (Kainantu) EHP	Afuan	New genotype		R. Peterson
	Niogamban, Western Prov.	Kalapua type (AAB)	New genotype		M. Weinert
	Lido Village, Sandaun	Indonesian banana (AAB or AAA)	0126	1	M. Weinert

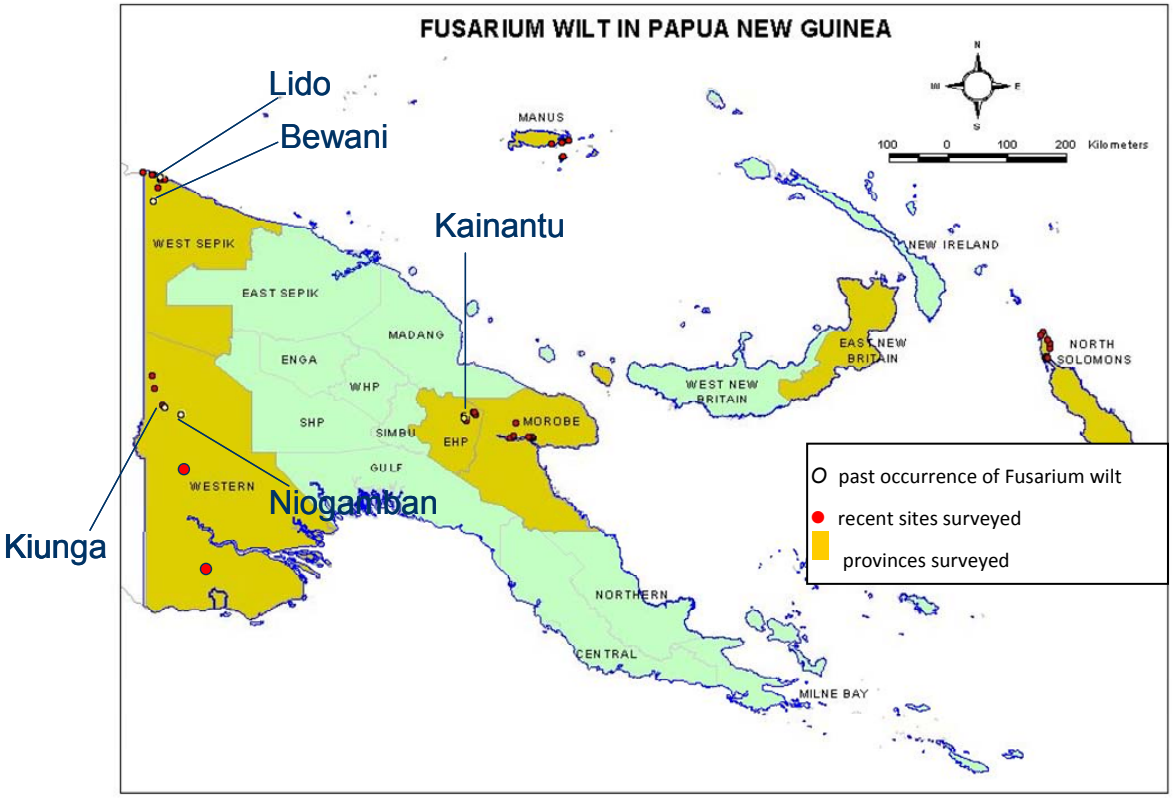

Figure 7. Surveyed provinces of Papua New Guinea

Major disease problems observed during the surveys included Sigatoka complex (including Black Cross, Cordana and Freckles) and *Marasmiellus* rot (Appendix 7). The widespread occurrence of *Marasmiellus* rot was also confirmed in recent surveys conducted by officers of the Northern Australian Quarantine Strategy (NAQS) and the PNG National Agricultural Quarantine and Inspection Authority (NAQIA) in Western (June 2008) and Sandaun Province (2007). During those surveys the team also did not detect any further *Foc* infections. However, villagers reported severe banana problems in more remote villages in Western Province.

A follow-up survey was conducted in May 2009 in Western Province because of reports of severe banana disease problems in remote areas of the province. The following sites in Western Province were visited: Hopnai Village, Matkomnai Village, Ningerum Station, Kiunga Town, Gwari CIS station, Samogos DPI station and South and Middle Fly Districts. From all the areas surveyed in Western Province, no diagnostic symptoms of banana Fusarium wilt disease were observed.

From all the surveys conducted, a total of 35 samples were collected and sent to QDPI for analysis. Based on the surveys conducted from the provinces of PNG, there were neither diagnostic symptoms of banana Fusarium wilt disease nor banana blood disease symptoms observed on plants in the villages. There were however other banana diseases observed such as: common banana leaf spot diseases and *Marasmiellus* rot.

The survey further confirms the concluded survey in 2008 conducted by plant pathologists. The surveys failed to observe typical symptoms of *Foc* on banana from the site where *Foc* Race 1 was first reported in Kiunga. For whatever reason the disease has disappeared or was not causing symptoms on plants at or within the vicinity of the site. It could be that the climatic conditions for disease development and spread were not conducive and suggested a specific survey on banana diseases be carried out in future during a dry season.

The 2008 border survey received reports of banana showing symptoms of Fusarium wilt and bacterial wilt at Tarakbits, and four other locations close to the border. The survey team could not visit the gardens because of time limitation. A separate survey was suggested where plant pathologists from PNG and Australia would spend at least a couple of days in suspected villages collecting samples for analysis in an overseas laboratory.

Banana blood disease and banana bunchy top are two other important quarantine diseases. It is just matter of time before they spread over to the PNG side of the border. In the case of bunchy top, it seems to be spreading through Indonesia over the last 20 years or so. There are no verified records of this disease on the island of New Guinea. However, there are very recent and very reliable reports that bunchy top disease-like symptoms have been seen in the north east of Papua, near Jayapura.

The farmers and the travelling public need to be informed of the risks of introducing Fusarium wilt (tropical race 4), banana blood disease bacterium and banana bunchy top virus.

Twenty nine new banana accessions were collected during the surveys and were added to the existing germplasm collection of NARI in Laloki. The accessions are currently being characterized and documented at NARI.

The PNG National Banana Germplasm Collection (NBGC) currently comprises of 221 accessions from many parts of the country. The collection is located at the NARI Dry Lowlands Programme at Laloki near Port Moresby. For the project, 10 varieties local varieties and 5 introduced varieties (Table 5) were selected initially from the NBGC based on their popularity as cooking bananas. All 15 varieties are also stored in tissue-culture in the International Transit Centre in Belgium. Lately it was also decided to include five ‘dessert-type’ international banana hybrids in the evaluation. The varieties are currently in the field for multiplication. They are currently multiplied using the banana bit technique to establish the first agronomic evaluation trial. It is anticipated that the evaluation trial will be established in Quarter 2 2010.

New varieties not yet contained and described in the NBGC were collected during the survey from different provinces. At the time of collection the number of banana suckers donated by farmers were one (1) to two (2) suckers only. The plants were rescued and potted in the nursery and later planted in the germplasm collection but due to a prolong dry period, plant growth was delayed and morphological characterization in the field including photographs is now expected to be completed in Quarter 2 2010 as well.

It is also planned to produce a Handbook of Common Banana Varieties. Varieties have been selected but data collection (agronomic data) and documentation (including photographs) may take time due to variability in maturity among the selected bananas.

Table 5. Banana varieties selected for evaluation

No.	Cultivar Number	Vernacular Name
1	PNG 155	Yawa
2	PNG 159	Maleb
3	PNG 161	Manameg red
4	PNG 101	Kekiau
5	PNG 206	Rukumamb Tambey
6	PNG 131	Daru
7	PNG 171	Dwarf Kalapua
8	PNG 168	Gunih
9	PNG 030	Babi
10	PNG 145	Kalapua No.2
11	FHIA 02	International hybrid
12	FHIA 17	International hybrid
13	FHIA 23	International hybrid
14	SH3436	International hybrid
15	Pisang Ceylan	International variety

** The PNG cultivars/accessions were selected on their popularity as 'cooking' bananas.*

***The international hybrids and varieties were selected on their resistance to Sigatoka leaf diseases and high bunch weights from a study under irrigated conditions at Laloki in 1999.*

7.1.3 Development of rapid molecular diagnostic tool

Conservation of *Foc* samples

All samples received have been placed in long term storage at DEEDI, Brisbane. As the isolates were characterised, they were transferred to long term storage by lyophilisation (freeze-drying). Pieces of agar plus carnation leaf are placed into vials under sterile conditions before being lyophilised in a Heto LyoLab 3000 freeze dryer. All 111 Indonesian isolates received as part of Hort/2005/136 and 67 samples received from Hort/2004/034 have now been freeze dried. Duplicate vials of each isolate are being held in cold storage under quarantine conditions at the Indooroopilly site.

DNA extraction

Three methods were investigated to extract DNA *from Fusarium oxysporum* f. sp. *cubense* samples. The methods investigated were phenol/chloroform extraction, Puregene kit (Qiagen) and a Biosprint kit. DNA was successfully extracted from the 111 samples received from Hort/2005/136 and 45 out 67 samples received from Hort/2005/034. The quantity of DNA extracted ranged from 1 ng/μL to ~ 350 ng/μL. The Puregene Yeast DNA extraction kit is able to extract high molecular weight DNA from *Foc* that is suitable for PCR and AFLPs. The method is transferrable to laboratories that have basic molecular equipment and culture equipment (water bath, centrifuge, sterile area/laminar flow).

TR4 PCR

A Tropical Race 4 PCR was developed by Bentley et al (1999). Each reaction consisted of the following (made up to a final volume of 25μL): 25ng DNA template; 1.5U *Tth* + DNA Polymerase (Fisher Biotech, Australia); 0.5μM of TR4F2 and 0.5μM TR4R1 (Table 1; Sigma Genosys, Australia); 0.2mM dNTPs mix, 3.0mM MgCl2, 1x PCR Buffer (Fisher

Biotech, Australia). Thermocycling conditions used were: 1 cycle of 95°C, 2 mins; followed by 30 cycles of 95°C, 30 secs; 68°C, 1 min 30 secs; followed by 1 cycle of 72°C, 3 mins. PCR product was run on a 1.5% agarose gel, post stained with ethidium bromide (10 mg/mL) and visualised under a UV light.

For positive samples, an amplicon of approximately 1400bp was observed, for negatives then no amplicon were detected (Figure 7). In the course of using this test, it was observed that an inconclusive result may be obtained for some isolates where duplicate testing did not match, i.e. one positive and one negative result, or where an amplicon of approximately 1400bp can be observed, but additional bands (or artifacts) are also present (Figure 8).

For project HORT2005/136, a total of 111 samples were tested. 56/111 (50.4%) tested positive for TR4. 47/111 (42.3%) tested negative and the remaining 7.3% (8/111) gave an inconclusive result. For project HORT2004/034 a total of 45 samples were tested. 19/45 (42.2 %) tested positive for TR4. 26/45 (57.8%) tested negative for TR4. Overall for both projects, 75/156 (48%) tested positive for TR4, 73/156 (46.8%) tested negative for TR4 and 8/156 (5.1%) were inconclusive.

Additionally, there appear to be some false positive (5/156 = 3.2%) and false negative (also 5/156 = 3.2%) results where VCG and TR4 PCR results don't match. If we assume that the VCG results are correct in these cases and ignore the inconclusive results, the PCR based assay has correctly detected 93.6% of the TR4 isolates from these projects. The use of these results should therefore be used with caution as it may have given incorrect results for approximately 6% of isolates, as well as a similar proportion of inconclusive results. The method is transferrable to laboratories that have basic molecular equipment (centrifuge, Thermocycling machine, and agarose gel electrophoresis equipment).

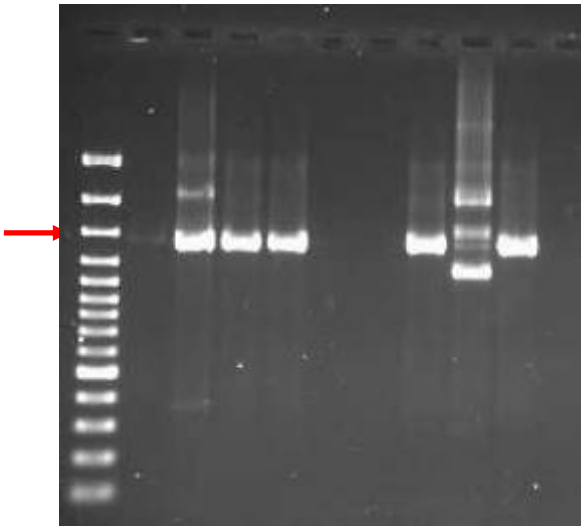

Figure 8. Tropical Race 4 results

M = DNA marker; lane 1 = faint positive result; lanes 2,3,4,7 and 9 show a good strong positive result, lanes 5 and 6 = true negative result, lane 8 = inconclusive - artefacts.

AFLP

AFLPs were successfully performed and analysed for 75 isolates, with 4 different primer combinations. DNA was extracted from selected isolates using the Puregene Gentra yeast kit (see above). After Sel PCR, a smear with distinct bands ranging between 700bp to 100 bp was visible for all samples processed. Often, different banding patterns could be observed for different samples processed with the same primer set. When the sel PCR product was run on the CEQ 8800, these bands were visible as distinct blue peaks with a maximum intensity of 200 000 units, whilst the size standard was visible as red peaks. A total of 427 polymorphic bands were identified across the four primers used, giving an average of 106 bands per primer set.

For ease of presentation, a combined phylogram (consisting of the 4 primer combinations) has been produced and presented (Figure 9). In general, isolates belonging to the same VCG, and isolated from the same cultivars tended to cluster together (Figure 9). For example, Clade IV, where 26/29 isolates in the cluster belong to VCG 01213/16 and 19/29 isolates were from the cultivar Barangan (Figure 3). Within this cluster was an isolate belonging to VCG 0121, which is in accordance with other studies that group 0121 with 01213/16.

It was expected that particular varieties would often yield the same VCGs due to plantations being established using 'dirty' planting material (and the particular cultivar acting as a 'vector') as opposed to disease free tissue culture planting material. However, VCG classification is only based on genes that govern heterokaryon formation, therefore this classification does not reflect the 'entire' genetic diversity of the organism - hence the reason that there may be some discordance between VCG and AFLP results. It is possible, due to mutations in the alleles governing heterokaryon formation (and hence VCG result), that isolates may be genetically similar, but have separated into different VCGs. A possible example of this can be seen in Clade XII of the combined phylogram where isolates 35 and 36 cluster together (very similar genetically) but belong to two different VCGs (0123 and 01218 respectively). A non-*Fusarium oxysporum* sample (4-2: *Fusarium semitectum* isolated from Siem) was included as an outlier and consequently formed its own clade with the least similarity to the *Foc* isolates (Figure 9).

AFLP has proven to be a useful tool for characterising *Foc* isolates, and relates well to VCG classifications. The AFLP method is not easily transferrable to other laboratories. Currently, QPI&F, Indooroopilly is the laboratory that has the equipment and personnel to perform AFLPs.

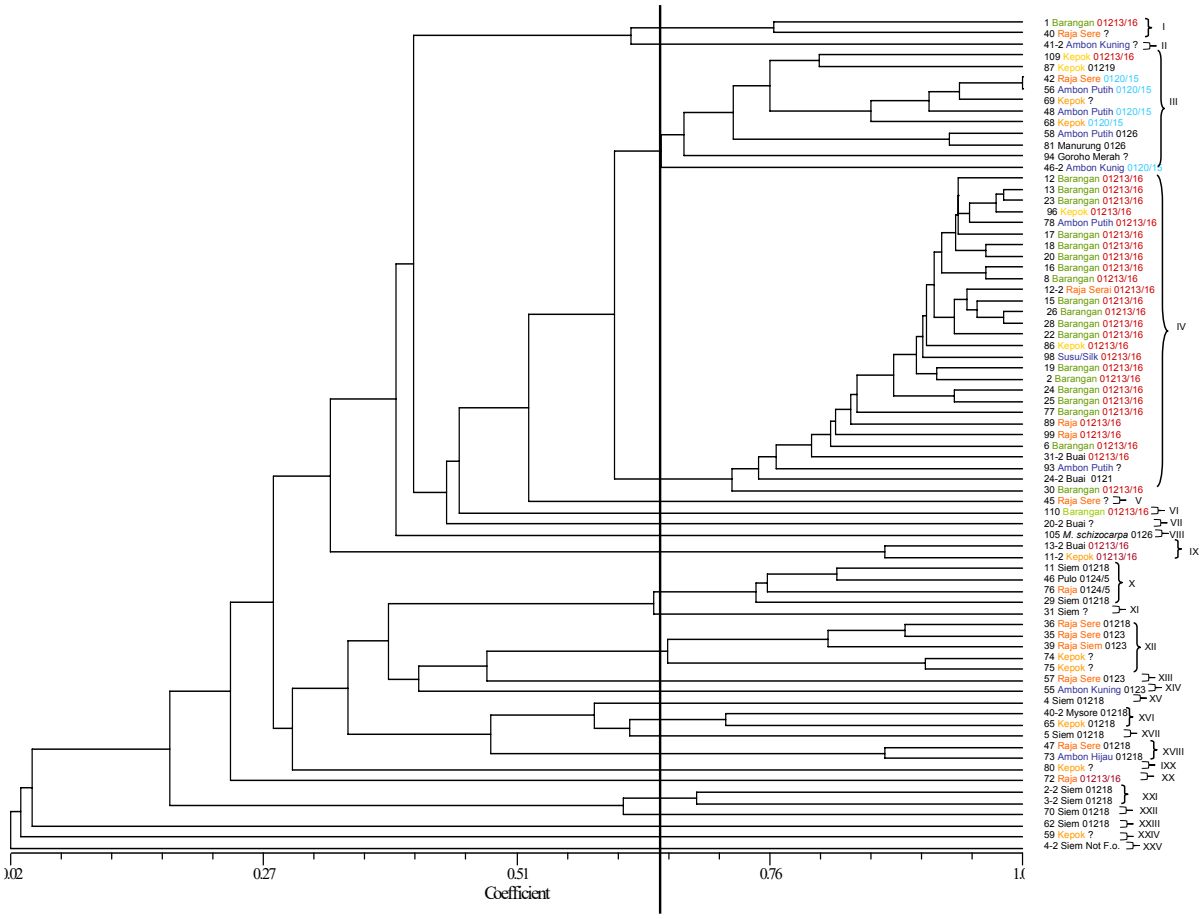

Figure 9. Phylogram of samples from AFLP analysis.

This phylogram represents combined data from 4 primer sets. A nominal value of 0.65 was used for clade distribution.

7.2 Virulence - Host Resistance Test

7.2.1 Indonesia

Both screenhouse and field virulence tests were conducted at ITFRI to understand the pathogenicity/virulence of various VCGs in Indonesia to different banana cultivars. This experiment was conducted with an effort to develop a “set of differential cultivars” that may serve as a diagnostic tool and guiding framework for disease management.

The following observations were made during the the screenhouse experiment:

- *Incubation period.* Observation was taken on the first observed external symptom, the first yellowing on the edge of leaf lamina. Incubation period is calculated from the time of inoculation to the first appearance of external symptom.
- *Disease severity of the leaf.* Daily observation was taken on the first sign of wilted leaves. Observation was stopped once all the leaves were yellowing or wilted and plant dies. The following formula of Mohammed et al (1999) was used to compute the disease severity on the leaf using the leaf symptom index (LSI). The scale on Table 6 and 7 were used to score the severity of the disease. Table 8 scaling system was used to translate the indices to their resistance category.

Table 6. Scale of leaf symptom index (LSI) (Mohammed et al. 1999)

Score	Criteria
1	no streaking or yellowing of leaves. Plants appear healthy.
2	slight streaking and/or yellowing of lower leaves (1-20% yellowing leaves)
3	streaking and/or yellowing of most of the lower leaves. Discoloration of the younger leaves may be just beginning to appear (21-50% yellowing leaves)
4	extensive streaking and/or yellowing on most or all of the leaves (51-100% yellowing leaves)
5	Plant die

Table 7. Rhizome discoloration index (RDI) (Jones, 1994)

Score	Criteria
1	Corm completely clean no vascular discoloration
2	initial points of discoloration in vascular tissue
3	discoloration of up to 1/3 of vascular tissue
4	discoloration between 1/3 and 2/3 of vascular tissue
5	discoloration greater than 2/3 of vascular tissue
6	total discoloration of vascular tissue

** Rhizome discoloration index (RDI) was observed destructively at the end of the observation*

After recording LSI and RDI, the overall disease severity index (DSI) for leaf symptoms and rhizome discolorations for each treatment was calculated as follows:

$$\textbf{DSI} = \frac{\sum (\text{number of scale x number of seedling that scale})}{\sum (\text{number of treated seedlings})}$$

Disease incidence was calculated using the following formula:

$$P = \left[\frac{T_1}{T_2}\right] \times 100\%$$

Note:

P = Disease incidence

T1 = number of diseased plant(s)

T2 = number of observed plant(s)

Table 8. Translation of DSI scales.

DSI scales for LSI	DSI scales for RDI	Resistance category
1	1	Resistant (R)
1.1 – 2	1.1 - 2	Tolerant (T)
2.1 – 3	2.1 - 3	Susceptible (S)
> 3	> 3	Highly susceptible (HS)

The LSI, RDI and DSI observations were taken from the test plants during the conduct of the field experiment.

Results showed Fusarium wilt symptoms[6] observed on inoculated plants. VCG 01213/16 was noted to be most virulent among the tested VCGs. These results indicate differences in disease resistance/ susceptibility of the various test varieties, and possibly differences in virulence of the different VCGs. Based on the evaluation conducted; all local varieties were found highly susceptible to VCG 01213/16 except the local variety Perancis (Table 9). The other cultivars are still being evaluated in the screenhouse for the remaining replications and further validation of the results.

'Pisang Rejang' displayed a mechanism of recovery when challenged to VCG 0124/5. The test plants died, but, then new healthy sucker was observed to have emerged at 30 days after inoculation. The sucker grew well and only showed LSDI of 1.86 that was categorized as tolerant (Figure 10).

To date, 15 cultivars were planted in the field (This study is going to be pursued in the second phase ACIAR project). The plants on the first block were eight months old, while at the second block the plants were two months old. Preliminary observations include the following: severe infection was found on Barangan, Ambon Hijau and Kilita indicating susceptibility of the varieties to TR4. Pisang Tanduk, Candi, Saba Awu, Kepok Kuning, Calcutta 4, and Karuk were found with no infection. Inconsistency with the result of the screen house test was specifically found on Kepok Kuning, Ambon Kuning, and Calcutta 4, where they found highly susceptible in screen house tests but resistant to tolerant at the field test level (Table 10 and Figure 11). This suggests a need to improve inoculation and assessment protocols in the screenhouse.

Table 9. Virulence of various VCGs to different local cultivars under screenhouse conditions

Banana varieties	**Species / genome**	*Foc* **VCGs**						
		01213/16	**0123**	**0126**	**01218**	**0124/5**	**0120**	**01219**
Calcutta 4	acuminata	HS	-	-	T-S	-	-	-
P. karuk	acuminata	-	S	S	R-T	R-T	HS	HS
Mas	AA	-	HS	HS	-	HS	-	HS
Rejang	AA	T-S	R	R	R	HS	T	T
Ambon Hijau	AAA	HS	S	T	T	HS	S	HS
Randah (Dwarf)	AAA	HS	T	T-HS	R-T	HS	T	T
A. kuning	AAA	HS	HS	HS	HS	HS	HS	HS
Barangan	AAA	HS	T	S	T	HS	T	T-S
Raja sere	AAB	T-S	T-S	T	T-S	HS	T	-
Raja bulu	AAB	HS	-	T	T-S	HS	T-S	-
Ketan	AAB	S	-	-	-	HS	T	-
Kilita	AAB	HS	HS	HS	S	HS	HS	HS
Kepok kuning	ABB	HS	HS	HS	HS	HS	HS	HS
Perancis	ABB	R	R	S-HS	R	HS	R	T

Notes: R = resistant, T = tolerant, S = susceptible, HS = highly susceptible

[6] such as vascular discolouration, chlorosis and wilting

Figure 10. Observed recovery mechanism of 'Pisang Rejang' at 30 days after inoculation when evaluated against VCG 0124/5. This could be a tolerance mechanism.

Table 10. Incidence and severity of Fusarium wilt on various varieties challenged by *Foc* VCG 01213/16, in screen house and field tests

Varieties/VCGs	Disease severity (%)				
	Screen house test		Screen house test		Field test
	External symptom-based (%)	Internal symptom-based (%)	LDSI	RDSI	
Karuk					
Barangan	96.60	96.60	4.60	5.62	100.
Ambon hijau	92.50	90.00	4.27	4.95	66.7
Ambon kuning	100.00	100.00	4.40	5.16	15.4
Kepok Kuning	100.00	86.04	4.01	3.40	0.00
Raja Bulu	78.00		5.38	3.51	66.7
Rejang	60.00	36.60	2.80	1.93	8.30
Mas	-	-	-	-	20.0
Perancis	0.00	0.00	1.00	1	0.00
Kilita	100.00	100.00	3.80	5.3	38.5
Calcuta	89.00	79.00	3.20	3.2	0.00
Berlin	-	-	-	-	25.0

Figure 11. Field pathogenicity test against VCG 01213/16 showing yellowing of leaves of the young plant (left) and vascular discoloration (right).

7.2.2 Papua New Guinea (PNG)

Fifteen banana varieties were selected for evaluation (Table 11). The PNG cultivars were selected based on their popularity as 'cooking' bananas. The international hybrids and varieties were selected on their resistance to Sigatoka leaf diseases and high bunch weights from a study under irrigated conditions at Laloki in 1999. They are currently multiplied using the banana bit technique to establish the first agronomic evaluation trial. It is anticipated that the evaluation trial will be established in the second quarter of 2010.

Table 11. Banana varieties selected for evaluation

No.	Cultivar Number	Vernacular Name
1	PNG 155	Yawa
2	PNG 159	Maleb
3	PNG 161	Manameg red
4	PNG 101	Kekiau
5	PNG 206	Rukumamb Tambey
6	PNG 131	Daru
7	PNG 171	Dwarf Kalapua
8	PNG 168	Gunih
9	PNG 030	Babi
10	PNG 145	Kalapua No.2
11	FHIA 02	International hybrid
12	FHIA 17	International hybrid
13	FHIA 23	International hybrid
14	SH3436	International hybrid
15	Pisang Ceylan	International variety

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7.3 On-farm disease management demonstration trials

The on-farm disease management trials *foc*used on production system improvement by adapting improved production and disease management practices that may improve banana yield and productivity. These were established in Dampit (East Java), and South Lampung (Figure 12). The elements of intervention included the use of disease-free planting materials in the form of tissue culture, resistant varieties, and cropping systems. Two introduced resistant varieties (FHIA 17 and FHIA 23) were compared to two popular local cultivars (Ambon Kuning and Ketan-01). These varieties were evaluated as monocrop compared to mix-cropping systems. In Dampit, some farmer cooperators interplanted the banana plants with nilam, coffee and cassava, the popular crops in this area. In Lampung, ground nut and ginger were used as intercrops. The specific practices discussed between the farmer representatives in the identified demonstration plots and researchers as represented by ITFRI were listed in Appendix 10.

Figure 12. Participatory planning workshop was conducted in Lampung and East Java

Figure 13. A workshop was conducted with local farmers introducing the banana in vitro plantlets

Figure 14. Participation of the local farmers; transferring hardened *in vitro* meriplants to polyethylene bags

The performance of banana demonstration plot in Dampit

Banana is known as one of main crops in Srimulyo after cacao and coconut. Farmers plant different varieties of bananas in the area. The most popular varieties are Ambon Warangan (Ambon Hijau; ripe-green Cavendish type), Kepok Kuning (ABB Saba type), Raja Talun dan Candi (plantain). Fusarium wilt infection is very important diseqase in this village and significantly reduced banana farmer income because most of the farmers in the area planted Ambon Warangan, a local variety susceptible to TR4. Currently, only 25% of the original banana plantation survive in the village.

Banana plants in Dampit were planted as multiple cropping mixed with *nilam*[7], coffee and cassava. Due to the problem of availability of planting materials, planting date could not be carried out at the same time. Progress of some demonstration plots in Dampit could not be followed up because the farmers changed their crops from banana to *nilam*, which was less risky and farmers get higher profits from the business than from banana production. In mixed cropping, farmers were more interested on the other crops thus often time neglecting the intercropped banana plants (Fig 15) especially those who used susceptible varieties. Some farmers who followed suggested cultural practices such as use of good seedlings and used proper spacing, fertilization and population management (desuckering) grew good size banana plants which have good stand for potential better yield. The final yield data were not assessed yet as of the writing of this report. Experiences in East Java suggest that in dealing with farmers the management of banana diseases should be a part of the whole cropping system. It is however important to identify communities which consider bananas as an important component of their production system hence their livelihood for a banana rehabilitation program to be succesful.

Figure 15. The performance of banana plants in a mix-cropping at the demonstration plot in Dampit, East Java

The performance of banana demonstration plot in Lampung

Farmers in Lampung were more receptive to improving their banana production because banana is an important crop of small scale farmers in this community. Like in Dampit banana plants were used in mixed cropping, with groundnut and ginger. The establishment of the field trial was similarly delayed because of delays in delivery of planting materials. At the time of this report, plants already 10-12 months old with some

[7] *Nilam is a herb (family Labiateae or Lamiaceae which produces atsiri oil)*

plants already harvested(Figure 16). Some plants of Ambon Kuning, a local variety but susceptible to Foc were already infected with Fusarium wilt. A few plants of Ambon Kuning were also infected by CMV (cucumber mosaic virus). The other disease that appeared in the demonstration plot was sigatoka. Pests have also been found in the plots, including stem borer, leaf roller and leaf eater caterpillar.

Preliminary data shows that FHIA 17 and FHIA 21,introduced resistant varieties from Bioversity International, showed no infection of Fusarium wilt compared to the local cultivars. Both FHIA 17 and 21 gave good agronomic and pathological performance. Average bunch weights were ranging from 22.4 and 28.7 kg. The high yield performance of the introduced varieties encouraged the farmer cooperator to try to use FHIA varieties in place of the local variety Ambong Kuning that is susceptible to Fusarium wilt. The only problem was the market. Since the varieties were introduced, the market responded poorly to the varieties and gave lower price (Rp. 600 – 800 = 60 – 800 cents per kg). To cope with the problem, it was suggested to the farmer to try processing the bananas to chips for adding value to his produce.

Intercrops initially showed less infection than the monocrops. The over-all performance of the various interventions are yet to be fully evaluated, however.

Figure 16. Field stand of bananas intercropped with ginger. Lampung, Sumatra

7.4 Training of researchers, extension workers, quarantine personnel for disease management and prevention

Training on banana soil health indicators and survey methodology was conducted last November 2006; Tony Pattison of DEEDI was the resource person. The training was conducted for the purpose of teaching ITFRI researchers on the theory of soil health, the use of soil health kit and establishment of the soil survey methods that was used for the preliminary characterization of soil samples in the surveys all over Indonesia. The participants who attended the training were trained on the use of the soil test kit which includes equipments to measure the following: sooil pH, electrical conductivity (EC), soil nitrate, soil temperature. The participants were also trained on how to do soil sampling in each survey site visited. The training was attended by 42 ITFRI researchers and field technicians (Figure 17). As an output of the said training, a user's manual was produced -- Soil Health Survey Manual.

Figure 16. Soil health training held in ITFRI Solok, West Sumatra last November 2006

The training for extension workers in PNG was conducted in Bubia, Lae last October 13 - 16, 2009. The workshop was attended by quarantine and extension officers in PNG (Figure 17). The training was conducted to increase PNG's ability for early detection, interception and incursion management of Fusarium wilt and other important banana diseases. Participants of the training were from the following: National Agricultural Research Institute (NARI), the National Agriculture Quarantine Inspection Authority (NAQIA) and the Department of Agriculture and Livestock. During the training, emphasis was given to the improvement of the capacity of extension officers to manage incursions of banana diseases into PNG with emphasis on measures to prevent the spread of Fusarium Wilt across PNG Indonesia Boarder. As banana is grown all throughout PNG and it is the fourth most consumed food with 34.3% after greens (75%), sweet potato (60.2%) and rice (35.1%). It is considered the most important staple crop with greatest production in Morobe, East New Britain, Central and Madang province.

The training highlighted the use of clean planting materials and resistant varieties as the primary means to prevent or limit the spread of the disease. The participants also identified factors that can contribute to the prevention of entry of *Foc* in PNG. Some of which that were enumerated where the following:

- the need to evaluate important PNG banana varieties for resistance to *Foc*,
- provide briefing papers on threat and potential impact of *Foc* and other diseases to policy makers,
- better coordination of emergency response to relevant agencies and development at all levels,
- formation of committees for further awareness raising in the provinces along the border through local level government and ward presidents, church leaders and teachers, and
- production of hand book of important banana disease.

Figure 17. Fusarium wilt quarantine training and workshop with quarantine personnel and researchers in Papua New Guinea held last October 2009

In Indonesia, the training was conducted based to bring together the collective expertise and most advanced technologies developed in the past 10 years. The training was conducted at ICHORD, Jl. Raya Ragunan 19 Jakarta on October 30 – 31, 2009 (Figure 18). The approach is to work with national agricultural systems in Indonesia and PNG and with banana farmers/growers on the ground in Indonesia, to build capacity and customize available technologies for use at local and national levels. The emphasis is on building coordinated national strategies for an already-affected country (Indonesia) and an urgently threatened country (PNG) that might be used as models for other countries in the region, while also bringing a range of measures directly into the field to allow the fast adoption of those that are effective.

The training was specifically conducted to build the capacity of national researchers and quarantine personnel in Indonesia and PNG to design and implement disease prevention and disease management measures for use against *Foc*. Researchers and quarantine personnel were trained in new improved diagnostics, pathogen characterization and selected disease management options.

The training workshop has attended by 15 participants from AIAT, Quarantine personnel, University, ITFRI, and ICHORD. Banana information and technologies encompassed production profile, farmer profile, pest status and distribution, varietal performance, sampling and isolation techniques, quarantine issues and regulations were delivered and discussed intensively. All the materials were provided for and distributed to participants at the end of the workshop for further extension and action. The workshop has also been enriched with a discussion on what the group going to do next, which resulted in the following commitments:

- Pests and diseases remain the main problem on banana cultivation; hence special attention should be addressed to this issue. ITFRI should provide distribution map of the pests that will be used for banana development purposes. A declared 'pest-free' area is needed to secure export confidence;

- The need to establish a “Banana rehabilitation program” that consists of rehabilitation of existing and new development areas.
- The need to change farmer perception from continuous (ever lasting) to three-harvest system of cultivation.
- The need to escalate banana consumption to anticipate increase of production. This can be achieved through food diversification. Promoting banana as part of the staple food is needed.
- Farmer and other agriculture-related organizations should remain vigilant to guarantee the system runs well,
- Collaboration among the institutions is an important aspect in capacity building, technological establishment, logistics preparation, information dissemination, and trading strategy development
- The participants will bring all materials and knowledge to engage local authorities in supporting a banana rehabilitation program. It was envisioned that every province will have a banana demo plot garden

Figure 18. Fusarium wilt quarantine training and workshop with quarantine personnel and researchers in Jakarta, Indonesia held last October 2009

8 Impacts

Through quarantine and management practices, the project will have immediate impact on the control of spread of Fusarium wilt within Indonesia, and on building national capacity to deal with outbreaks of different strains of the disease, and on preventing its entry into new areas, such as PNG.

8.1 Scientific impacts – now and in 5 years

The research outputs of the project provide preliminary understanding of the diversity and pathogenicity of Fusarium wilt against various local and introduced banana cultivars in Indonesia; these findings have implications for banana research and production worldwide, and for new information integrated into existing knowledge about this important disease. Molecular characterization of the various isolates has provided more information and knowledge in developing tools that are essential in managing the disease, such as in the areas of diagnostics, gene deployment and breeding.

The extensive collection and conservation of *Foc* isolates strengthened the regional and international *Foc* reference collection library in DEEDI and ITFRI. These will be available for broader upstream studies on *Foc* by various researchers worldwide.

The results indicating pathogenic interaction between VCGs and various varieties opens up new opportunities to elucidate the concept of pathogenic Races in Foc.

8.2 Capacity impacts – now and in 5 years

Several collaborative extension activities were conducted in Indonesia and PNG. The research and extension capacities of the involved institutions were enhanced through collaborative workshops and trainings from the project in disease diagnosis, sample collection and processing, and via field management trials and laboratory analyses, including VCG analyses.

The countries involved in the project had significant increase in capacities to deal effectively with outbreaks of different strains of Fusarium Wilt and to prevent its incursion into new areas.

8.3 Community impacts – now and in 5 years

The project will have immediate impact on the control of the spread of the disease through quarantine and management practices, enhance the national capacity to deal with outbreaks of different strains of Fusarium wilt and to prevent its incursion in new areas.

Direct participation of farmers in demo-trials allowed them to learn and apply new knowledge in disease management and banana production as well, thus empowering farmers at the community level.

8.3.1 Economic impacts

Economic benefits of increased farmers' income will be realised in terms of:

- reduced yield losses on commercial plantations and smallholder farms,
- reduced losses due to disease,
- lower labour costs
- increased yield brought about by the adoption of improved varieties and management practices evaluated in conjunction with farmers

8.3.2 Social impacts

The project was expected to benefit smallholders, as well as more commercial farmers. Smallholders' natural and human capital in project sites should increase, as a result of carrying out participatory evaluation of practices, resistant varieties and other disease management tactics. The project aimed at promoting improved practices and varieties through extension mechanisms and via farmer association and field learning .

8.3.3 Environmental impacts

Farmers will be encouraged to improve and use integrated management practices for the control of the disease and thus will have an impact on sustainability of *Musa* cultivar diversity, and land and crop management. The project had collected new varieties which with further evaluation and improvement maybe useful in increasing diversity in varieties used by farmers therby promoting stability and ecosystem sustainability

The project will encourage the adoption of exotic disease-resistant varieties and may consequently present as yet unquantified threats to the conservation of indigenous diversity.

8.4 Communication and dissemination activities

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9 Conclusions and recommendations

9.1 Conclusions

Fusarium wilt a major banana disease limiting banana production Indonesia. Tropical Race 4, and other strains of Foc are now widely found affecting bananas in almost all provinces in Indonesia. Survey results corroborate that banana disease is the main production constraint. The project's findings highlight the widening distribution of TR4 *Foc* in Indonesia, and its spread and apparent predominance over other VCGs since previous surveys were conducted, which emphasises the importance of managing its spread to other areas within Indonesia and neighbouring countries. Infected planting material continues to be moved to new areas (within and between provinces), and TR4 is now found very close to the border of Papua New Guinea (PNG) in Papua Province (Davis et al., 2000), posing an imminent threat to the extensive banana diversity of that country. It was also found that Blood disease, a bacterial wilt disease is equally destructive as Fusarium wilt. Project surveys have provided a wealth of data on the current status of banana pests and diseases in the region ,and farmers will be more able to deal these pests and disease problems as a whole rather than individually. Disease management field trials participated in by farmers indicated that management of diseases is part of the whole production system and even influenced by market. Most farmers considered banana production in a mixed-cropping system. While the use of clean banana seedlings were important component of the disease management-crop production system, their availability and affordability at farm level was very important. While resistant varieties were available and proved effective in reducing incidence of infections, and also were higher yielding, market acceptance is a very important consideration for their adoption. The adoption of re-integration of bananas in the cropping system of farmers was also affected by its profitability relative to other crop options. The general survey however, still showed that bananas are important source of foods and livelihoods of many small scale farmers. Managing banana diseases to improve livelihoods of farmers should be approached in cropping system strategy with the overall goal of improving their income.

Results indicate that high nitrate levels in acid soils may be conducive to Fusarium development, and highlighted some interactions between soil pH, electrical conductivity (EC) and nitrate levels as factors influencing disease levels. However further studies are needed to adequately understand the role of soil health and properties in the incidence and suppression of *Foc* on banana (see below).

Enhanced local capacities of researchers, extension workers, quarantine personnel and growers will facilitate more effective disease management.

9.2 Recommendations

Foc is already widespread in Indonesia, thus managing banana wilts should be a major component of a cropping system improvement. For PNG, prevention of incursion of banana wilts, and creating readiness to contain these diseases should be the focus.

Papua New Guinea Survey

Further surveys are recommended: A) conducted specifically during the dry season in Kiunga to address concerns in the previous survey regarding lack of apparent *Foc* symptoms in the area. It is hypothesised that such lack of symptoms may be attributed to non-conduciveness of conditions ; B) conducted along the Indonesia-PNG border to address reports of *Foc* incidence in Tarakbits. Collection of samples was not made in these areas due to time limitation.

Soil health studies

Further studies are recommended to adequately understand the role of soil health and properties on the incidence of *Foc* on banana. These recommended studies should measure a greater number of soil parameters that account for physical, chemical and biological soil properties. The observed variations of infection of *Foc* in some locations may suggest that suppressive soil exist and there is a need to understand this mechanism to improve abilities to mage Foc through biological means.

Molecular Characterization

It is recommended that further studies be conducted to identify rapid TR4 molecular characterization methods that is transferrable to well equipped molecular laboratories in developing countries with problems in banana TR4. The use of PCR in characterizing TR4 should be used with caution as it may have given incorrect results for approximately 6% of isolates, as well as a similar proportion of inconclusive results. The method is transferrable to laboratories that have basic molecular equipment. On the other hand, the use of AFLP has proven to be a useful tool for characterising *Foc* isolates, and relates well to VCG classifications, however, the AFLP method is not easily transferrable to other laboratories.

Virulence-Host resistance test

Field pathogenicity tests of potentially resistant or tolerant varieties based on the screenhouse tests and experimental field tests has to be conducted in TR4 infected areas in Indonesia. The field evaluation of the varieties has to be replicated and should be well documented.

Training recommendations

The training highlighted the use of clean planting materials and resistant varieties as primary means to prevent or limit the spread of the disease. The participants identified factors that can contribute to the prevention of entry of *Foc* in PNG:

- the need to evaluate important PNG banana varieties for resistance to *Foc*,
- provide briefing papers on threat and potential impact of *Foc* and other diseases to policy makers,
- better coordination of emergency response to relevant agencies and development at all levels,
- formation of committees for further awareness raising in the provinces along the border through local level government and ward presidents, church leaders and teachers, and
- production of hand book of important banana disease.

The following commitments have been drafted in the workshop as well:

- ITFRI should provide distribution map of the pests that will be used for banana development purposes. A declared 'pest-free' area is needed to secure export confidence;
- The need to establish a “Banana rehabilitation program” that consists of rehabilitation of existing and new development areas.

- The need to change farmer perception from a continuous to a three-harvest system of cultivation.
- The need to promote increased banana consumption to help stimulate an increase in production. This can be achieved through food diversification. Promoting banana as a part of the staple food is needed.
- Farmer and other agriculture-related organizations should remain vigilant to help guarantee the system runs as well as possible,
- Collaboration among the institutions is an important aspect in capacity building, technological establishment, logistics preparation, information dissemination, and trading strategy development
- The participants will bring all materials and knowledge to engage local authorities in supporting a banana rehabilitation program. It was envisioned that every province will have a banana demo plot garden

Demonstration plots

Further evaluation and field verification of Fusarium wilt management practices and options will be continued in the next ACIAR project (HORT 2008/040). Demonstration plots will be established in Lampung and Cianjur provinces. The over-all performance of the various interventions is yet to be evaluated. This should be approached in a cropping systems improvement approach.

To prevent Fusarium spread, farmers and the travelling public need to be informed of the risks of introducing Fusarium wilt (tropical race 4), the banana blood disease bacterium and banana bunchy top virus.

It is also planned to produce a Handbook of Common Banana Varieties. Varieties have been selected but data collection (agronomic data) and documentation (including photographs) may take time due to variability in maturity among the selected bananas.

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11 Appendices

11.1 Appendix 1: Percent appearance of identified *Foc* VCG on each province*

Province	Percent appearance of identified *Foc* VCG on each province								
	01213/16	0120	0121	123	0124/5	0126	01218	01219	Total
Nangroe Aceh Darussalam	22.22	0.00	0.00	0.00	0.00	0.00	3.27	0.00	25.49
West Sumatera	14.38	0.00	2.61	0.00	0.00	0.00	2.61	1.31	21.57
Yogyakarta	5.88	3.92	0.00	0.65	0.00	0.00	1.96	1.31	13.73
North Sulawesi	4.58	0.00	0.00	0.00	0.00	0.00	0.00	1.31	5.88
Lampung	4.58	3.92	0.00	0.00	0.00	0.00	0.00	0.00	8.50
West Kalimantan	2.61	0.00	0.00	0.00	0.00	0.00	1.96	0.00	4.58
North Sumatera	2.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.61
East Java	1.96	0.65	0.00	0.00	0.00	0.00	0.00	0.00	2.61
West Java	1.31	1.31	0.00	1.96	0.65	0.65	1.96	0.00	7.84
Southeast Sulawesi	1.31	0.00	0.00	0.00	0.00	0.65	0.00	0.00	1.96
East Kalimantan	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65
Papua	0.65	0.00	0.00	0.00	0.00	0.65	0.00	0.00	1.31
Central Kalimantan	0.00	0.65	0.00	1.31	0.00	0.65	0.00	0.00	2.61
South Kalimantan	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.65
Total	62.75	10.46	2.61	3.92	0.65	3.27	11.76	3.92	

*Note: * calculated from 153 VCG results*

Samples collected from South Sulawesi are not finished

11.2 Appendix 2: Percent appearance of VCG on total identified isolates on each banana variety

Variety	Percent appearance of VCG on total identified isolates								
	01213/16	0120	0121	0123	0124/5	0126	01218	01219	Total
Barangan	24.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.84
Kepok	4.58	1.31	0.00	0.00	0.00	1.31	1.96	2.61	12.42
Ambon Hijau	7.19	0.00	2.61	0.00	0.00	0.00	0.00	0.00	9.80
Raja	12.42	0.65	0.00	0.00	0.00	0.00	0.00	0.00	13.07
Ambon Kuning	4.58	5.23	0.00	0.65	0.00	0.65	0.00	0.65	11.76
Awak	0.65	0.00	0.00	1.96	0.00	0.00	6.54	0.00	9.15
Rajasere	1.31	2.61	0.00	1.31	0.00	0.00	2.61	0.65	8.50
Unknown	1.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.31
Nangka	0.65	0.65	0.00	0.00	0.00	0.65	0.00	0.00	1.96
Rojomolo	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65
Panjang	1.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.31
Tanduk	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Candi	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65
Jantan	1.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.31
M.Schizocarpa	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.65
Manis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cavendish	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65
Goroho Merah	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mauli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mysore	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.65
Pulo	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.65
Randah	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rejang	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65
Total	62.75	10.46	2.61	3.92	0.65	3.27	11.76	3.92	100.00

**Ambon Putih = Ambon Kuning*

Ambon Hijau = Buai

Kepok = Manurun, Sepatu, Cepatu

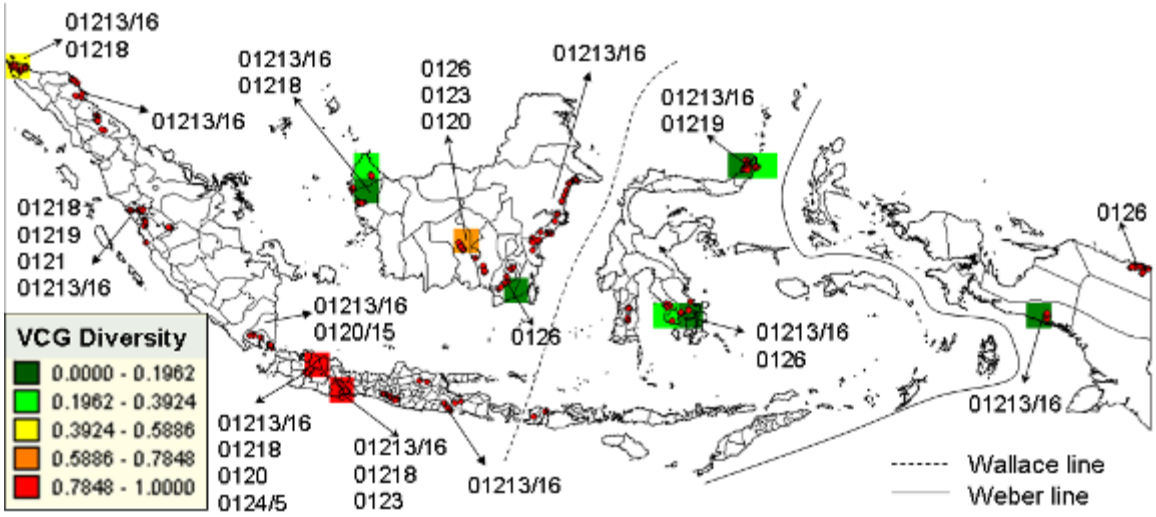
Barangan = Gapi

Siem = Awak, Serawak, Raja Siem

Raja = Raja Bulu

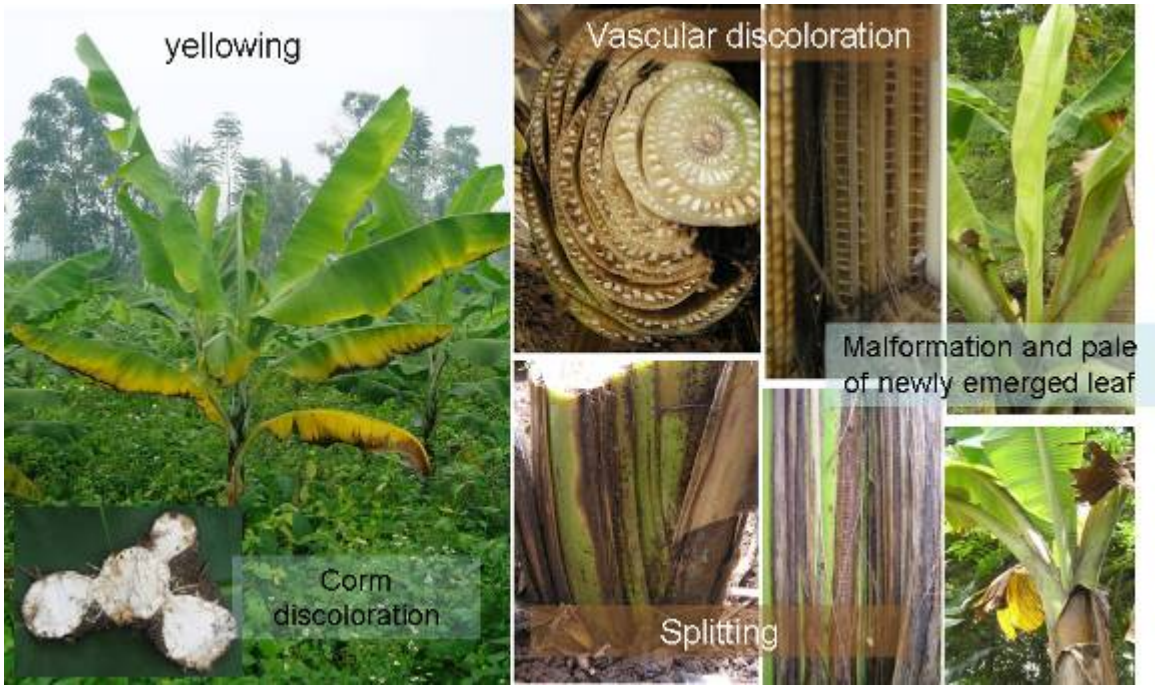
Jantan = Ketan, Uli

11.3 Appendix 3. Progressive map of the diversity of *Foc* VCG in Indonesia



Note: Red colour showed by higher value of diversity index indicates higher VCG diversity (more VCG were found)

11.4 Appendix 4. Pictures of commonly found banana diseases and pests in Indonesia



Fusarium wilt, the major complaint of banana farmer

Blood disease, another major complaint

Banana weevil, the pest that farmer thinking to be the causal agent of wilting

New threat, banana bunchy top disease, initially endemic in West Java and Lampung provinces, found to be spread up in almost all provinces.

New threat, bacterial corm rot (*Erwinia sp.*), newly emerging deadly disease but was found rare

Wild *Musa schizocarpa* was found Fusarium wilted (identified as VCG 0126) in the road side around 10 km from the border of Jayapura and Papua New Guinea.

11.5 Appendix 5. Banana pests and diseases found in five main islands of Indonesia

No	Pests and Diseases	Sumatera	Java	Kalimantan	Sulawesi	Papua
1	Fusarium wilt	++ to +++	++ to +++	+	++	+
2	Sigatoka leaf spot	++	++	+	++	++
3	Cordana leaf spot	+	+	+	++	+++
4	Black cross disease	-	+1)	-	++	+++
5	Giant banana leafspot	+2)	-	-	-	-
6	Blood disease	+++	+++	+++	++	++3)
7	Bacterial corm rot	+	+	+	pe	+
8	BBTV	++	+++	+	+	+
9	Mosaic disease	+	pe	-	+	+
10	Nematode	+	+	pe	+	pe
11	Leaf roll4)	+ to +++	+ to +++	+ to +++	+ to +++	n
12	Thrips5)	+	+	pe	+	+
13	Fruit scab	+ to ++	+ to ++	+ to ++	+ to ++	-
14	Fruit fly	+	pe	-	pe	-
15	Stem borer	+ to +++	+ to ++	+	+ to ++	pe
16	Corm borer	+	+	+	+	pe
17	Leaf eater weevil	-	+6)	-	-	-
18	Leaf eater caterpillar	+	+6)	-	-	-
19	Fire caterpillar	+	-	-	+	-
20	Red and white caterpillar	-	+1)	-	-	-
21	Plant hopper	-	-	-	-	+
22	Hemiptera	-	-	+7)	-	-
23	Mites	Pe	+	-	-	+
24	Termites	-	-	+	-	-
25	Nutrient deficiency	Pe	pe	+	pe	-
26	Herbicide phytotoxic	-	-	pe	-	+
27	Soil inundation	-	+	+	pe	+

+, ++, +++ = consecutively from rare to frequently found

- = not found during survey

pe = pest or case was not found during survey but predicted exist in the area

11.6 Appendix 6. Banana varieties observed during the surveys in Indonesia

Name			Synonyms
Variety	Genome	Subgroup	
Berlin	AA	Berlin	Muli, Maoli, Bawang, Burung, Nona, Lampung
Mas Bunga	AA	Berlin	
Mas	AA	Sucrier	
Rejang	AA	Cv Rose	
Jarum	AA	Cv Rose	
Sario			
Lilin	AA	Lilin	
Kapas	AA		
Rakit	AA		
Cavendish Williams	AAA	Cavendish	
Morosebo	AAA	Dwarf Cavendish	
Ambon Hijau	AAA	Giant Cavendish	Buai, Ambon Lumut, Ambon Hong
Ambon Kuning	AAA	Grosh Michel	Ambon Putih
Barangan	AAA	Barangan	Ayam, Gapi
Udang	AAA	Green/Red Banana	
Rojomolo	AAA	Green/Red Banana	Embug, Rojomolo
Raja Bulu	AAB	Raja Bulu	Raja
Raja Serai	AAB	Silk	Susu
Nangka	AAB	Nangka	
Ketan	AAB		Jantan, Uli, Ketip
Raja Lilin	AAB	Mysore	
Goroho	AAB/AA?		
Goroho Merah	AAB/AA?		
Talas	AAB/AA?		
Tanduk	AAB	Horn Plantain	
Candi	AAB	False Plantain	
Tanduk Merah	AAB	Horn Plantain	
Lampeneng	AAB		Slendang
Kastroli	AAB?		Goroho Gajah?
Pulo	AAB?		
Kelat	AAB?		
Longok	AAB?		
Janek/Koyut	?		
Caras	?		
Pisang panjang			
Pisang-pisang			
Kepok	ABB	Saba	Batu, Sepatu, Cepatu, Nipah, Menurun, Manurung, Sanggar
Kepok Besar/Jawaka	ABB		
Kepok Tanpa Jantung			
Awak	ABB	Awak	Uwak, Siem, Raja Siem, Uter, Raja Banyu, Pulau Pinang, Batu
Sobo	ABB	Bluggoe	Sepatu Putih

Name			Synonyms
Variety	Genome	Subgroup	
Klutuk (*Musa balbisiana*)	BB	Klutuk	Batu
Wild#1 (*Musa acuminata*)	AAw		
Wild#2 (*Musa acuminata*)	AAw		
Wild#3 (*Musa acuminata*)	AAw		
Wild#4 (*Musa acuminata banksii*)			
Wild#5 (*Musa scizocarpa*)			
Musa Bornensis			

11.7 Appendix 7. Major Banana diseases recorded in PNG (Davis 2000; Davis et al. 2001; Davis et al. 2000; Shaw 1984)

Common name	Scientific name
Diamond leaf spot	*Cordana musae*
Pseudostem rot	*Marasmiellus semiustus*
Leaf spot	*Cladosporium musae*
Anthracnose of fruits	*Colletotrichum musae*
Black Sigatoka	*Mycosphaerella fijiensis*
Sigatoka disease	*Mycosphaerella musicola*
Black cross	*Phyllachora musicola*
Banana streak virus	*Badnavirus*
Root knot nematode	*Meloidogyne incognita*
Burrowing nematode	*Radopholus similis*
Panama Disease	*Fusarium oxysporum 'Race 1'*

11.8 Appendix 8. List of banana varieties evaluated for the pathogenecity test

No	Species/ Group	Subspecies/ Subgroup	Name	Code	Origin
1	Acuminata	Burmanicoides	Calcutta 4	ITC 0249	INIBAP
2	Acuminata	Sumatrana	P. karuk		West Sumatra
3	Balbissiana	Klutuk	Klutuk		West java
4	AA	Jari Buaya	Rotan		West Sumatra
5	AA	Mas	Mas		East java
6	AA	Berlin	Muli/Berlin		Lampung
7	AA	Cv. Rose	Rejang		West Java
8	AA		Kaikeja		Papua
9	AAA	Cavendish	Ambon Hijau		West Sumatra
10	AAA	Cavendish	Randah (Dwarf)		West Sumatra
11	AAA	Gros Michel	Ambon Kuning		West Java
12	AAA	Barangan	Barangan		North Sumatra
13	AAB	Silk	Raja sere		North Sumatra
14	AAB	Raja	Raja bulu		West Java
15	AAB	Jantan	Ketan		West Sumatra
16	AAB	Plantain	Tanduk		East Java
17	AAB	Plantain	Candi		East Java
18	AAB	Plantain	Kilita		Papua
19	ABB	Saba	Kepok kuning		West Sumatra
20	ABB	Saba	Jawaka		Maluku
21	ABB	Awak	Raja siem		West Java
22	ABB	Bluggoe	Sobo Awu		East Java
23	ABB	Bluggoe	Raja Kinalun		Bengkulu
24	AAAA		FHIA-25	ITC1418	INIBAP
25		Fei	Tongkat langit		Papua

11.9 Appendix 9. List of VCGs from Indonesia evaluated for the pathogenecity test

No	VCG	Isolate code	Origin and banana variety
1.	01213/16	01.02.02.02	NAD / Barangan
2.	0123	02.01.02.02	West Java/ Siem
3.	0124/5	02.02.01.14b	West Java/ Pulo
4.	01218	02.02.01.15	West Java/Rajasere
5.	0120	02.02.01.26	West Java/Ambon Putih
6.	0126	05.01.02.01b	South Kalimantan/ Kepok
7.	01219	001/NS	South Sulawesi/ Kepok
8.	0121		West Sumatera/Ambon Hijau

11.10 Appendix 10. Banana production methodologies agreed between the researchers (ITFRI, QDPI&F, AIATs) and the representative farmers of the demonstration plots

Technologies	Lampung	Malang	Best bet package
Farmer cooperator	Yusuf Samad and Solihin	Farmer group	
Cropping system	Mixed cropping with cacao and monoculture	Mixed cropping with 'nilam'	Monoculture
Variety	Ketan	Ambon Warangan (Ambon Kuning), Kepok, Raja Talun	FHIA 17 FHIA 21 Ambon Kuning (Lampung) Ketan (Malang)
Planting material	Sucker, 50 cm height	Sucker, 50 cm height	Tissue culture
Planting space	3 x 3 m	3 x 3 m	3 x 3 m
Organic manure	10 l/planting hole, once a year	10 l/planting hole, once a year	10 l/planting hole, once a year
Inorganic fertilizer	None	Yes	Yes
Weeding	Using herbicide, twice a year	Manual	Manual
Bedding	Twice a year	Twice a year	Twice a year
Desuckering	Yes; 3 plants per mat will be maintained	Manual	Manual and kerosene application
Deleafing	Yes	Yes	Yes
Deflowering	Yes	Yes	Yes
Bagging	No	No	Yes
Management of dying/infected and death plant	Dug, chopped, and burned with dried leaves	Burned on site	10 ml of Glyphosate injection Burned on site with rice hull
Status by May 2008	Land preparation During the miniworkshop conducted on 27 November 2007, the farmers from both sites were introduced with hardened plantlet of Barangan (beyond the listed varieties for demoplot). They successfully organized the planting materials and planted in their farm. Confidence with the first performance of the farmer in handling the planting materials, ITFRI delivered hardened plantlets for the demoplot to the farmer group, letting them having more experience with tissue culture material, but the result of the second chance is very poor, the mortality of the seedling is very high. The existing planting materials are not enough to set up the demoplot. Imperative for ITFRI to prepare ready-to-plant banana planting materials to cover the failure of the previous materials; will be ready to plant by September 2008.		
Status by February 2009	All varieties agreed with farmer in both location (Lampung and Malang – East Java) were planted, but the number of plants is not enough yet. ITFRI gradually supply the planting materials until the required number is fulfilled Some plants at Lampung site were suspected to be infected by bacterial wilt. Early-selected eradication were done by farmer using rice husk burning Demoplot in Malang – East Java: farmers are now less interested to banana anymore (after the outbreak of blood disease in this area) due to there is other crop (nilam) that gives more income than banana. The demoplot (with less interest) is now located at three farmers, intercropped with maize.		

11.11 Appendix 11. List of private laboratories producing banana tissue culture plantlet

NAMA LABORATORIUM	ALAMAT	TELEPON/FAX	VARIETAS
BBU Wonocatur, Yogyakarta		0274 517004	
BBI Salaman, Magelang		0293 335270	
Mariwati, Cipanas			
Caringin			
PT Darul Falah (Tekno Agro Mandiri), Bogor	Jl. Raya Bogor – Ciampea km 12 Bogor	0251 420416 Susi: 08129612175	
Fitotek, Jakarta		021 - 7271444 Elda (0811994066)	
PT Tamora, Medan			
Puslit Kopi dan Kakao, Jember Jl. PB Sudirman 90, Jember 68118 Jawa Timur Telp: 0331-757130, 757132, 757065 Fax: 0331-757131	Ir. Heri Purwanto Kepala Urusan Pemasaran Rumah: Jl. Hayam Wuruk XIX No. 208, Jember Telp: 0331-426199 HP: 08124936218	0813	Kepok Kuning, Barangan, Raja Bulu Pemesanan di luar ke tiga varietas tersebut harus dilakukan dengan batas minimal 20 ribu bibit
BBI DKI			
Politani,	Cibeduk - Ciawi	Suharyati: 0251 – 315780	
Biotrop	Jl. Raya Tajur Km 6 PO Box 116, Bogor 1600	0251 323848 0251 356077	
Inagro - Parung		0251 - 541500	
BBI Gedung Johor - Medan		Herawati: 085270694916	
BBI Ragunan	Jl. RM Harsono No 1 Ragunan, Pasarminggu, Jakarta Selatan	021 7805236	
BBI Salaman	Jl. Raya Magelang – Purworejo Km 16, Menoreh, Salaman, Magelang, Jawa Tengah	0293 335270	
BBI Sedau	Jl. Pemepek – Menemeng, Kab. Lombok Tengah, Pringgarata, NTB	0370 671710	
BBI Gedung Johor	Jl. Jendral Besar AH Nasution No 6 Gedung Johor, Medan, Sumatera Utara	061 7863567	
BBI Sidera	Jl. Lasosso Km 14, Desa Sidera, Kec. Sigi Biromaru, Donggala, Sulawesi Tengah	Dinas Pertanian Sulawesi Tengah atau BPSBTPH Sulawesi Tengah 0451 482774	
Diperta Propinsi Jambi	Jl. RM Noor Admadibrata, jambi 36122	0741 60717 0741 62404 Fax: 0741 62829	
Diperta Propinsi Riau	Jl. Raya Pekanbaru – bangkinang km 8, Pekanbaru	0761 61054 Fax: 0761 61052	
Prof. Dewa Ngurah Suprapta,	Lab. Biopestisida, Faperta Universitas Udayana,	0361 222450 Fax: 0361 255346	

NAMA LABORATORIUM	ALAMAT	TELEPON/FAX	VARIETAS
	Kampus Jimbaran, Badung Bali		
PT Bumi Teknokultura Unggul	Jl. Kapten Tendean No 11 Kuningan Barat, Jakarta Selatan	021 5274358-9 Ir. Elda Adiningrat (Direktur)	

11.12 photos of symptoms:

11.12.1 Appendix 12: Wilting plantlets of Ambon Kuning (left tray) and Barangan (right tray) one month after inoculation in VCG-01213/16

11.12.2 Appendix 13. A) External symptom of *Foc* TR4 (VCG-01213/16) on Ambon Kuning and Barangan and B) transverse section of the infected corms.

11.12.3 Appendix 14. External and internal symptoms of VCG-0123 on Barangan; A) wilting plant, B) transverse section of the corm and pseudostem showing discoloration of pseudostem (red arrow), and C) cross section of the corm