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Australian Centre for
International Agricultural Research

Project annual report

project

Mitigating the threat of banana Fusarium wilt: Understanding the agro- ecological distribution of pathogenic forms and developing disease management strategies

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LIST OF ACRONYMS

ACIAR	Australian centre for International Agricultural Research
DNA	De-oxyribonucleic acid
ENB	East New Britain Province
FHIA	Fundación Hondureña de Investigación Agrícola (banana cultivar prefix)
Foc	<i>Fusarium oxysporum</i> f.sp. <i>cubense</i>
GCTCV	Giant Cavendish Tissue Culture Variant (a code name for a somaclonal variant variety)
ITFRI	Indonesian Tree Fruit Research Institute
NARI	National Agricultural Research Institute of PNG
PC	Partner Country
PNG	Papua New Guinea
QDPI&F	Queensland Department of Primary Industry and Forestry
TC	Tissue Culture
TR	Tropical Race
VCG	Vegetative Compatibility Group

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1 Progress summary

Our project objectives, regarding mitigating the threat of banana Fusarium wilt remain the same.

1.1 Survey, Collection and Characterization

1.1.1 Indonesia

The survey and collection of samples within the framework of the CP/2005/136 project is complete in eight major Indonesian banana producing provinces (See Section 2). One hundred and eleven *Fusarium oxysporum* f.sp. *cubense* (Foc) pure isolates were sent to QDPI&F for VCG (Vegetative Compatibility Group) and DNA characterization. The Foc isolates came from 16 different banana varieties.

To fully map the Foc distribution in Indonesia, provinces under the Indonesian / ACIAR bilateral project (CP 2004/034) are being surveyed to complement surveys under project CP/2005/136. ITFRI is generating pure isolates from the samples collected, to be sent to QDPI&F for VCG and DNA characterization.

VCG analyses at QDPI&F show that 73 out of the 111 isolates gave positive VCG results, belonging to several VCG groups (See Section 2). 44 out of the 73 VCG-positive isolates showed specific results for VCG 01213/16, the VCG that is associated with the virulent Tropical Race 4 (TR4). The remaining 38 isolates were negative in the VCG tests. There was a confirmation of VCG 0126 on a wild banana, *Musa schizocarpa*, collected from Papua Province. Nine new *Musa* accessions were collected from Central Kalimantan, North Sulawesi and Papua.

1.1.2 Papua New Guinea (PNG)

Survey and collection activities are being conducted in PNG. Six field surveys, yielding 36 samples were completed between September 2007 and February (See Section 2) These were sent to QDPI&F for testing. *Fusarium* wilt-like symptoms were uncommon in these surveys, indicating that *Fusarium* wilt disease is not yet well established in PNG. QDPI&F tests show that none of the 36 samples from PNG was infected with *Fusarium* wilt disease. Survey and collections are scheduled for June 2008 in Sundaun, which borders with Papua, where TR4 has been previously reported.

A huge diversity of local bananas was observed in Manus (See Section 2).

Taxonomists from the survey-team collected new banana accessions from Manus (10) and Western province (6). New germplasm accessions are important potential sources of resistance for specific banana diseases, including Foc TR4. The accessions were added to the existing germplasm collection of NARI in Laloki.

1.1.3 Development of rapid molecular diagnostic tool

A key project objective is to develop an accurate and rapid diagnostic molecular technique test for *Fusarium* wilt. One hundred and two Foc-positive isolates from Indonesia were studied by extracting their DNA, using a commercial DNA plant extraction kit¹. Tests

¹ BioSprint Tissue Lyser

showed that 47 samples were found to be positive for TR4, 35 to be negative, and 20 samples gave inconclusive results. The accuracy of the test on the 102 samples was computed at 80.39%, which is relatively low, thus test procedures will yet have to be refined.

1.2 Virulence-Host Resistance Test

Four local banana varieties, Ambon Kuning, Barangan, Kepok, and Raja Sere, have been initially tested for resistance to the six following VCGs (See Section 2): 01213/16, 0123, 0120, 01218, 0126, and 012415. The project will be conducting virulence tests on a total of 25 selected wild and cultivated banana varieties to eight VCGs (See Section 2). Preliminary results showed Fusarium wilt symptoms² were observed on inoculated plants. Other tested local varieties showed common symptoms such as yellowing and wilting of leaves even three months after inoculation. Despite some observed corm infection. VCG01213/16 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.

1.3 On-farm disease management demonstration trials

The farmer co-operators participatory planning workshop assessed farmers' practices in banana production and disease management, as well as options for management of Fusarium wilt disease. From this workshop, the following are the 'best-bet' options for Fusarium wilt management: The use of:

- disease-free planting materials obtained from tissue culture (TC) propagation;
- varieties tolerant or resistant to Fusarium wilt such as FHIA-17, FHIA-21, FHIA-25, GCTCV 119, Ketan-01, and one susceptible variety Ambon Warangan (Cavendish subgroup);
- burning rice-hull on infected mats or spot eradication;
- good agronomic practices;
- a biological control using *Pseudomonas fluorescens*, adopted from the farmers' field-school program;

Demonstration plots were established in Lampung, Central Java and Malang, East Java in cooperation with farmer-co-operators.

The farmers were trained on the use of clean planting materials from banana tissue culture and the management of tissue culture seedlings for field planting. One important aspect of the project is to introduce a sustainable TC delivery system to small-scale farmers sourcing from commercial laboratories. Private tissue culture laboratories (22) producing banana meriplants were identified across Indonesia. A meriplant delivery system and a management protocol for village nurseries is being developed.

² such as vascular discolouration, chlorosis and wilting

2 Achievement 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	November 2007	Surveys were completed in eight major banana producing regions/ provinces of Indonesia: Aceh, West Java, West Kalimantan, Central Kalimantan, South Kalimantan, North Sulawesi, Southeast Sulawesi and Papua
				Seven VCG groups were identified from analyses of the samples from Indonesia. VCG 01213/16, the VCG associated to Foc Tropical race 4 were found from samples from the provinces of Aceh, West Kalimantan, Southeast Sulawesi and North Sulawesi. Other provinces such as West Java, West Kalimantan, South Kalimantan and Papua have other Foc VCGs such as 01218, 0123, 0120, 0124/5, 0126 and 01219.
				The following VCGs were isolated from the following local varieties: VCG01213/16 from the varieties' Barangan, Rajabulu, Ambon Putih, Raja and Kepok; VCG01218 isolated from the varieties' Rajasere, Ambon Hijau, Siem, Nangka and Kepok; VCG0123 isolated from Rajasere, Ambon Kuning and Siem; VCG0120 isolated from the varieties' Rajasere and Ambon Putih; VCG0124/5 was only isolated from Pulo; VCG0126 was isolated from M.schizocarpa, Ambon Putih, Kepok and Manurun; VCG01219 was isolated only from Kepok

no.	activity	outputs/ milestones	completion date	comments
			May 2008	To fully map the extent of Foc distribution in Indonesia, several provinces of the country under the Indonesian – ACIAR bilateral project (CP 2004/034) project are currently being surveyed to complement the initial surveys conducted under the project CP 2006/136. Nine additional provinces were identified for the survey, namely: Aceh, North Sumatera, West Sumatera, Lampung, West Java, Yogyakarta, East Java, South Sulawesi and East Kalimantan. Surveys have been completed in West Sumatera, Yogyakarta and East Kalimantan. To date, ITFRI is in the process of generating pure isolates from the samples collected. Once characterized, a geographic map of Foc distribution in Indonesia based on Foc VCG and varieties affected shall be developed.
			February 2008	Survey and collection activities were carried out in PNG, a total of 6 field surveys were conducted from September 2007 to February 2008 namely in: (1) Morobe Province/ Markham Valley, (2)Kainantu, (3)North Solomon Province/ Buka, (4) East New Britain Province (ENB), (5)Manus and the (6) Western Province. From the surveys conducted, a total of 36 samples were collected and sent to QDPI&F for VCG analysis
				No Fusarium wilt symptoms were observed on banana plants in ENB and Manus, PNG. Other diseases observed in the area are Marasmiellus rot caused by Marasmiellus inoderma, sigatoka, black cross, cordana leaf spot and leaf speckle
				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
				From the Western Province, a total of 6 unidentified banana varieties were also collected. The varieties are being held in Post Entry Quarantine at NAQIA. All the plants collected are being regenerated and will later on be characterized for further identification.

no.	activity	outputs/ milestones	completion date	comments
		National/ regional pathogen specimen reference collection expanded	May 2008	111 pure isolate -Foc samples from ITFRI were sent to QDPI&F for VCG and DNA characterization.. These isolates are being stored as a part of the regional Foc library maintained at QDPI&F. The isolates were maintained in Carnation leaf agar, then lyophilized in a Het LyoLab 3000 freeze dryer for long term conservation. No Foc isolates were recovered from PNG samples
1.2	Enhance and validate molecular characterization tools	Refined molecular tools for identification and characterization of VCGs/ races	May 2008	Commercial DNA extraction kit was used to extract the DNA of Foc isolates. 102 samples have been tested using TR4 PCR, 47 samples were positive for TR4, 35 samples were negative and 20 samples showed inconclusive results. The accuracy of the test was computed at only 80.39%, which is deemed low. Further optimization of the technique is to be pursued. DNA fragments of Foc have been generated from the isolates but not all fingerprints are readable. Fingerprints are also not reproducible, thus requiring further optimization.

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	May 2008	<p>Twenty five banana accessions composed of wild and cultivated, diploid, triploid and tetraploid banana representatives maintained at ITFRI genebank were selected for the study</p> <p>Four varieties, Ambon Kuning (AAA), Barangan (AAA), Kepok (ABB), and Raja Sere (AAB), have been initially inoculated to six VCGs, namely, 01213/16, 0123, 0120, 01218, 0126, 012415. Inoculations on tissue culture test plants were conducted in screenhouse at ITFRI. Severity of internal and external symptoms was recorded as measures of virulence/ resistance. This initial test is an effort to optimize the protocols for the study. Preliminary results showed Fusarium wilt symptoms such as vascular discoloration, yellowing of leaves and wilting, were observed on inoculated plants. The variety 'Barangan' was severely infected by VCG 01213/16. All 'Barangan' test plants died within 5 – 11 days after incubation. Other tested local varieties such as Ambon Kuning, Kepok and Raja Serai inoculated with VCG 01213/16 only showed common Fusarium wilt symptoms such as yellowing and wilting of leaves even until 3 months after inoculation despite some observed corm infection. VCG01213/16 was noted to be most virulent among the tested VCGs.</p>

PC = Partner Country, A = Australia

Objective 3: To develop and validate disease management tactics appropriate to the severity of local disease forms, varieties grown and local 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.		<p>Bioversity, QDPI&F and ITFRI conducted a participatory planning workshop with farmers and local extension agents in Lampung and Malang in June 2007. The workshop was conducted to initially assess the farmers' practices to manage banana diseases and to introduce to the farmers the use of disease free planting materials. These are now being carried out in both locations</p> <p>From the participatory workshop with farmer-co-operators, the following are the ‘best-bet’ options for Fusarium wilt management: (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.</p>

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		Demo-trial plots were established in Malang and Lampung with farmer co-operators. Some farmers used a combination of the above options, while others did not. These are being monitored by the local extension agents' partners. Due to insufficient planting materials some farmers are yet to establish their own field trial. ITFRI is preparing the planting materials.
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		A survey was carried out to identify existing private banana tissue culture laboratories that are producing clean banana seedlings which could be tapped as sources of affordable planting materials for small scale farmers. Twenty-two private tissue culture laboratories producing banana meriplants were identified in Jakarta, Bogor, Bali, Sulawesi, Java and Sumatra. A system of meriplant delivery system and a management protocol for village nursery is being developed.
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		The soil-diagnostic kit to determine root-soil health conditions were used during the survey. Data were collected from all areas surveyed, as part of the associated variables for each sample area. These data will be used in the overall analyses of the survey results.
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		(None for this report period. Scheduled for the third year)

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		(none in this reporting period. This will be done in the third year)

3 Impacts

Through quarantine and management practices, the project will have immediate impact on controlling the spread of Fusarium wilt; 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.

3.1 Scientific impacts

This research will provide a fundamental understanding of the diversity and pathogenicity of Fusarium wilt that will have implications for banana research and production worldwide. New isolates will be fully characterized, and new information integrated into existing knowledge about this important disease. Molecular characterization of the various isolates will provide more information and knowledge in developing tools that are essential in managing the disease, such as in the areas of diagnostics, and gene deployment and breeding.

The extensive collection and conservation of Foc isolates will strengthen the regional and international Foc reference collection library in QDPI&F. These will be available for broader upstream studies on Foc by various researchers worldwide.

The pathogenic relationships between VCGs and various varieties will be established, an important contribution to the current understanding of Races and VCGs

3.2 Capacity impacts

Indonesia and PNG research and extension capacity will be enhanced through collaborative research activities from the project and through training on disease diagnosis, sample collection and processing, and via field management trials and laboratory analyses, including VCG analyses.

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

3.3 Community impacts

Through quarantine and management practices, the project will have immediate impact on the control of the spread of the disease and 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 allows them to learn and apply new knowledge in disease management and banana production as well, thus empowering farmers at the community level.

3.3.1 Economic impacts

Economic benefits 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

3.3.2 Social impacts

The project is 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 aims to promote improved practices and varieties through extension mechanisms and via farmer association and agricultural field days.

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

3.4 Communication and dissemination activities

Bioversity International – Asia-Pacific Office (Philippines). 2007. RISBAP Bulletin (Newsletter). Volume 11, No. 3 (July - September 2007). Banana Asia-Pacific Network (BAPNET), Bioversity International, Asia-Pacific Office. Los Baños, Laguna, Philippines. 8pp.

Bioversity International – Asia-Pacific Office (Philippines). 2008. RISBAP Bulletin (Newsletter). Volume 12, No. 1 (Jan - March 2008). Banana Asia-Pacific Network (BAPNET), Bioversity International, Asia-Pacific Office. Los Baños, Laguna, Philippines. 4pp.

Gulino, L., O'Neill, W., Pattison, A., Daniells, J.W., Williams, R. and Molina, A. 2007. Update: Fighting Fusarium TR4 in Indonesia and Papua New Guinea. Australian Bananas 25: 46 – 47

Gulino, L., W. O'Neill, C. Hermanto, A. Molina and A. Pattison. 2007. Fusarium wilt of bananas in Indonesia and Papua New Guinea. Abstract submitted for presentation at the 9th International Congress of Plant Pathology on 24 – 29 August 2008 in Torino, Italy.

Gulino, L., W. O'Neill, C. Hermanto, A. Molina and A. Pattison. 2007. Fusarium wilt of bananas in Indonesia and Papua New Guinea. In Back to Basics: Managing Plant Disease. Proceedings of the 16th Biennial Australasian Plant Pathology Society Conference held at the Adelaide Convention Centre, 24 – 27 September 2007. Adelaide, Australia.

Presentations:

Jeff Daniells, on the various banana pest and disease threats on our doorstep in Indonesia including Foc TR4, to the Innisfail Banana Growers Association, May 2007.

Wayne O'Neil, on general aspects of the ACIAR project CP 2006/136 and also his experiences from the South East Sulawesi field survey, at the University of Queensland Fusarium seminar series, December 2007

Jeff Daniells, on aspects of his travel to the International Banana Crop Protection Symposium in South Africa with emphasis on Foc to the Innisfail Banana Growers Association, February 2008

Gus Molina, citing the ACIAR project on Fusarium wilt, presented “Challenges facing the banana industry and their impact on trade”, to the FAO/CFC China Workshop on Opportunities and Challenges in the World Markets for Fruits and Tropical Products held at the Trade Promotion Centre, Guangzhou, China, November 29-30 2007.

Gus Molina, citing the ACIAR project activities, presented “Recent occurrence of *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 in Asia”, to the International Society of Horticultural Science - ProMusa Symposium on Recent Advances in Banana Crop Protection for Sustainable Production and Improved Livelihoods. Greenway Woods Resort, White River, South Africa, September 10-14, 2007

4 Training activities

Training on handling of tissue cultured banana seedlings and banana tissue culture nursery management was conducted in November 2007, at Lampung and Malang, East Java. Twenty-two farmers and 5 local extension workers from Lampung attended the training workshop, while 13 farmers of the group 'Bumi Mulyo' and 3 local extension workers attended from Malang.

5 Intellectual property

(No issues to report for this period)

6 Variations to future activities

A mid-project review workshop is scheduled for August 2008. This workshop was not planned in the project document, hence unbudgeted. However, the project team believe that it is necessary to meet at this point to review the progress of the project against our objectives and timelines. Our next steps will also be agreed upon towards a successful delivery of outputs. This review workshop is also relevant in view of some project personnel changes and addition. This workshop will also be timely with the terminal project review of CP/2004/134, the results of which will be useful for the final completion of CP/2005/136. The budget for this workshop will be drawn from some savings on other activities deemed less important. A request for reallocation of budget has been submitted through the ACIAR project manager.

7 Variations to personnel

Jayne D. Generoso, Administrative and Finance Officer of Bioversity International in the Philippines took the place of Versalynn Roa

Ma. Lizbeth Severa J. Baroña, Communications Specialist of Bioversity International in the Philippines took the place of Jeffrey Oliver

8 Problems and opportunities

The survey activities within the framework of CP 200/034 were delayed, hence the map of VCG distribution for Indonesia is not yet complete for this reporting period. The responsibilities of this project had been transferred to our partners in CP 2006/136 to enhance the realization of this activity.

9 Budget

As reported in the separately submitted ACIAR acquittal for the reporting period December 2007 to 31 May 2008, out of AUS \$31,309 receipts, AUS \$7,395 was spent or 23.6% of the funds received. The total budget for the reporting period is Aus \$148,361, and the apparent under-spend can be accounted for by the different phasing of the QDPI, NARI, NAQIA and ITFRI LOAs.

We will conduct a mid-term project review-workshop in Indonesia in August. This workshop was not originally planned and budgeted, but ACIAR have approved a re-allocation of Aus \$ 9,000 from the Contracted services to go to Travel/Subsistence to fund the travel expenses for project participants and coordination travels. The balance of the Contracted Expenditures will be used for the technical assistance required for the field demo-plots in Indonesia.