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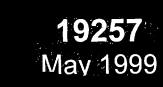
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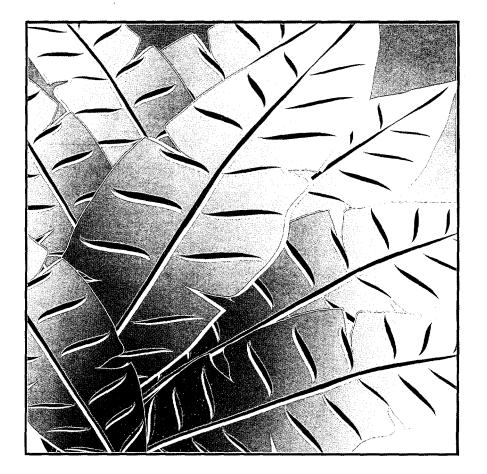
ENVIRONMENTALLY AND SOCIALLY SUSTAINABLE Development Agricultural Research and Extension Group Series

Banana Improvement Project Report No. 2



Banana, Breeding, and Biotechnology

Commodity Advances through Banana Improvement Project Research, 1994–1998



Edited by Gabrielle J. Persley Pamela George ENVIRONMENTALLY AND SOCIALLY SUSTAINABLE DEVELOPMENT AGRICULTURAL RESEARCH AND EXTENSION GROUP SERIES Banana Improvement Project Report No. 2

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Foreword

ver 80 million metric tons of banana and plantain are grown throughout the tropics. About 10 percent of the total crop, mainly dessert bananas, is exported, with a value of approximately US\$5.3 billion. The balance is either sold locally or grown as an important subsistence food crop.

Dessert bananas grown for export are vulnerable to a range of diseases which impact on the cost, production levels, environment, and the health and safety of the workers involved in the industry. Despite the importance of the commodity and decades of diligent research, banana breeding has had little success in producing the desired results of new disease-resistant dessert banana varieties suitable for the commercial trade. This was in part due to the lack of suitable techniques to facilitate genetic improvement in banana. This fact, and the limited linkages between commercial banana production/handling and publicly funded research programs, prompted the World Bank and its partners to initiate in 1994 a unique five-year programthe Banana Improvement Project (BIP).

The World Bank is grateful to the Common Fund for Commodities (CFC) and the FAO Inter-Governmental Group on Bananas (FAO/ IGB) for co-sponsoring this important endeavor. CFC generously funded the research projects by providing approximately US\$3.5 million over five years to sponsor the portfolio of projects reported in this document. These funds leveraged substantial additional technical and financial support for banana research, primarily from national, regional and international public sources.

Innovative structuring, focused goals, and carefully selected individual research projects, with secure funding and clear time-lines, were the basis for success. BIP attracted some of the most talented scientists in the world to tackle, the intractable problems of breeding dessert bananas resistant to damaging diseases and pests, using sophisticated research methodologies and the new tools of biotechnology.

The new knowledge generated through BIP on disease and pest resistance and the new enabling technologies for the genetic improvement of banana is critical to the future viability of the industry, and to the improved livelihoods of resource-poor banana producers. The Project results will also contribute to the improved environment and health status of workers through reduced use of chemicals. This book documents these achievements. The independent economic assessment of BIP (see chapter 4) indicates a likely return on this research investment of 20 to 33 percent.

The World Bank encourages other international development agencies, commercial companies, producer co-operatives and other parties concerned with the production and trade of banana and plantain and the livelihoods of those who grow them to take a close look at the new opportunities offered in future banana research and development initiatives.

> Alexander McCalla, Director Rural Development Division (RDV) The World Bank

Acknowledgments

he World Bank gratefully acknowledges the intellectual and practical contributions of all principal investigators, colleagues, and students who worked on the BIPsponsored research projects. It is their scientific contributions that have resulted in new strategic knowledge important to banana growers worldwide. The World Bank thanks those involved in arrangements for hosting the three annual scientific meetings at their institutes, Katholieke Universiteit Leuven (KUL), Belgium, Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement and Departement des Production Fruitieres et Horticoles (CIRAD/FLHOR) France and Guadeloupe, and Centre de Recherches Regionales sur Bananiers et Plantains (CRBP), Cameroon.

The many hours devoted to the selection and monitoring of the Project by the members of Scientific Advisory Panel is particularly appreciated. Their combined expertise and dedication contributed significantly to the design, execution and the success of the Project. The membership of the Panel over the course of the Project is listed in Appendix 2. The World Bank also thanks the International Plant Genetic Resources Institute and its International Network for the Improvement of Banana and Plantain (IPGRI/INIBAP) for the participation of the INIBAP Director at the meetings of the Scientific Advisory Panel.

The World Bank would like to extend its appreciation to the other cosponsors: the CFC, for their investment of financial resources and the provision of technical and management support to the Project; and FAO and its Intergovernmental Group on Bananas, which formed a Project Technical Advisory Committee, to support the planning and execution of the Project. The contributions of World Bank staff responsible for the technical and financial management of the project are also gratefully acknowledged.

The technical editor, Mr. Reginald MacIntyre, and Alicia Hetzner (Managing Editor for the Environmentally and Socially Sustainable Development Network at the Bank), provided valuable assistance throughout the writing and production of this book. .

Acronyms and Abbreviations

ACP	African, Caribbean and Pacific (states)
BADC	Belgium Administration for Development Cooperation
BBTV	Banana bunchy top virus
B/C Ratio	Benefit-cost ratio
BIP	Banana Improvement Project
BBMV	Banana bract mosaic virus
BLSD	Black leaf streak disease
BSV	Banana streak badnavirus
BTI	Boyce Thompson Institute for Plant Research, USA
CATIE	Centro Agronomico Tropical de Investigacion y Ensananza
CFC	Common Fund for Commodities, The Netherlands
CIRAD	Centre de Cooperation Internationale en Recherche Agronomique pour le
	Developpement, France
CRBP	Centre de Recherches Regionales sur Bananiers et Plantains, Cameroon
CITA	Centro de Investigacion y Technologia Agrarias, Canary Islands
CORBANA	Corporacion Bananera Nacional, Costa Rica
EMBRAPA	Empresa Brasiliera de Pesquisa Agropecuaria, Brazil
FAO/IGB	Food and Agriculture Organization of the United Nations,
	Inter-Governmental Group on Bananas, Italy
FLHOR	Departement des Productions Fruitieres et Horticoles, CIRAD, France
FHIA	Fundacion Hondurena de Investigacion Agricola, Honduras
IITA	International Institute of Tropical Agriculture, Nigeria (CGIAR)
INIBAP	International Network for the Improvement of Banana and Plantain,
•	France (CGIAR)
IPGRI	International Plant Genetic Resources Institute, Italy (CGIAR)

x Acronyms and Abbreviations

IRR	Internal rate of return
IRTA	Instituto de Recerca i Tecnologia Agroalimentaries, Spain
KUL	Katholieke Universiteit Leuven, Belgium
MGIS	Musa Germplasm Information System
NPV	Net present value
PCR	Polymerase chain reaction
PNG	Papua New Guinea
QDPI	Queensland Department of Primary Industries, Australia
QTL	Quantitative trait loci
QUT	Queensland University of Technology, Australia
R&D	Research and development
RFLP	Restriction fragment length polymorphism
VVOB	Flemish Association for Development Cooperation and Technical
	Assistance, Belgium
UNDP	United Nations Development Programme

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Summary and Conclusions

"Careful channeling of funds to precisely targeted research objectives with the right programs will lead to new, useful knowledge and to new profitable products. New innovative breeding approaches combined with advances in plant biotechnology offer really new dimensions to banana improvement."

The Banana Improvement Project has in large measure met the challenge above, quoted from the 1994 background paper by Buddenhagen (1996) on the status of global research on banana and plantain. That paper was commissioned by the World Bank to provide background for the five-year BIP program that brought together some of the best research talent worldwide to work on bananas. BIP operated throughout 1994-98, and this publication records the results of the 18 individual research projects, plus the results of an independent economic impact assessment that reported a likely return on investment of 20 to 33 percent. This uniquely conceived and innovatively run program has achieved its main goals. The Chair of the BIP Scientific Advisory Panel at the Third Scientific Meeting in November 1998 observed that

BIP-funded research projects have made exciting discoveries, contributed considerable useful new knowledge, and established strong collaboration and linkages that will continue into the future. Important advances have been made in the understanding of the genetics of resistance to black Sigatoka. We now have a much greater understanding of what we face with the Fusarium threat. Molecular tools to transform bananas have been developed, and key banana breeding programs have been strengthened as an investment in problem solving. Resistance to nematodes is now better understood, and we have in hand the means of controlling some significant virus diseases of banana. BIP has also demonstrated that chemical control strategies aimed at managing resistance to fungicides of black Sigatoka will not work. We also now know that tissue culture apparently predisposes plants to Fusarium and nematodes.

BIP, through the project researchers, has delivered many significant scientific advances. The work has produced an excellent return on investments, and all principal investigators and collaborators are to be commended for their outstanding work. Substantial advances have been achieved in Musa research, but we must build on these successes. Future programs need to focus on the most promising results and pressing issues to maximize benefits of the research, which should be based on a market-driven *approach.* The diversification of products to provide choices for diversified demand patterns should be available, and should meet the criteria of consumer markets as well as improve the livelihoods of the banana producers, traders and workers throughout the world.

The structure of BIP, its management, and competitive grant style of selecting and funding individual research projects, are described below, together with the details of the research results.

Background

Banana and plantain are grown mainly by smallholder farmers in developing countries. Bananas are of major importance to food security in the tropics, and their production provides a vital source of income through local and international trade. Banana and plantain are the fourth most important global food crop and export commodity. They rank first as a fruit.

Only in very recent years has research been able to improve banana breeding techniques, thus overcoming some of the barriers to genetic improvement of this crop. Producers are still facing serious pest and disease problems, which are controlled in large measure by the use of chemical sprays. Resource-poor farmers often cannot afford expensive pesticides, so they suffer damaging losses. The use of pesticides is a major health concern in developing countries, so the development of disease- and pestresistant varieties is viewed as urgent to protect a vital industry and the health of banana producers.

A significant contribution to this global effort has been made through BIP, an international five-year research program that used some innovative scientific and management approaches, including a competitive grants structure, to harness the talents of outstanding researchers. BIP started in January 1994 and contracted research concluded in December 1998, with some of the individual research projects continuing their activities into 1999 for completion of their work.

The Project was cosponsored by the Common Fund for Commodities (CFC), the FAO Inter-Governmental Group on Bananas (FAO/ IGB), and the World Bank. CFC committed funding of SDR2.5 million (approximately US\$3.5 million) to the project portfolio and management of the program. The World Bank, as the Project Executing Agency, prepared a detailed completion report covering the management and research activities resulting from this investment. The report was presented to the cosponsors, along with a financial statement, individual project completion reports, and the impact assessment document.

This publication is a distillation of all of the documentation, highlighting the important outcomes and impacts of the research funded by BIP. Although BIP has made significant advances, more work needs to be done using the latest tools of biotechnology. The BioBanana program outlined in Chapter 5 aims to accomplish this when it is fully funded and operational.

BIP had two main objectives:

1. To develop and evaluate improved banana varieties with export potential, which would

incorporate increased productivity and durable disease resistance through conventional and nonconventional breeding techniques

2. To develop more efficient and integrated disease management practices, especially for black Sigatoka disease.

BIP funded 18 research projects in four broad technical areas: (a) biotechnology, (b) germplasm collection and characterization, (c) plant improvement, and (d) pest and disease management. The intended beneficiaries were producers of dessert bananas, particularly smallholders. The potential spillovers to producers of plantain for subsistence were increasingly recognized during the course of the Project.

Project Structure

Management

The cosponsors and their technical representatives constituted the governing body of the Project, with FAO/IGB as the Supervisory Body in accordance with CFC normal practices. In order to meet their obligations to CFC, FAO convened a Technical Advisory Committee to assess project progress. The World Bank acted as the Project Executing Agency, handling the daily management of the project, and convened a Scientific Advisory Panel (SAP) to assist in the planning, selection, and monitoring of research activities. The Bank reported to CFC and FAO/IGB on project activities with twice-yearly Progress Reports, and Annual Reports. Annual Meetings of the Cosponsors were held to discuss project progress, and met at the same time and venue as the Annual Scientific Meetings.

Research

The eighteen projects were chosen through a merit-based process of internationally competi-

tive research grants. Proposals were reviewed by SAP, and those selected formed a cohesive and complementary portfolio to work in parallel toward BIP objectives. Projects were monitored by SAP on a six-month basis through review of Progress and Annual Reports. When projects were recommended for acceptance by the project executing agency, principal investigators were notified of the review outcome. Annual Scientific Meetings permitted principal investigators to present their work to the cosponsors, their technical panels, and scientific peers.

Research Highlights

Results of research activities are set out below against the two BIP objectives, followed by results against technically related activities.

Results against BIP Objectives

To develop and evaluate improved banana varieties with export potential, which would incorporate increased productivity and durable disease resistance through conventional and nonconventional breeding techniques.

- Refined and utilized two methods of genetic transformation
- Genetically engineered expression of resistance to two viruses: banana bunchy top virus (BBTV) and banana bract mosaic virus (BBMV) into Cavendish and other varieties
- Collected and preserved valuable germplasm, and biotypes of nematodes and pathogens
- Reversed serious decline in conventional breeding capacities in two regions
- Contributed to the safe international transportation of important genetic stocks
- Supplied breeding programs with useful diploid lines as parental stocks.

To develop more efficient and integrated disease management practices, especially for black Sigatoka disease.

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- 4 Banana, Breeding, and Biotechnology
- Expanded options for pest management choices by:
 - Identifying important components of durable host plant resistance
 - Adding understanding to the consequences of pesticide use
 - Identifying sources of resistance in germplasm collections
 - Mapping genetic resistance to the black
 Sigatoka pathogen
 - Completing a global map of geographical distribution of *Fusarium* races for risk assessment
 - Developing an early screening protocol for nematode-resistant varieties.

Results against BIP Technically Related Activities

Area 1: Biotechnology. BIP succeeded in its strategy to develop a molecular toolbox. Methods for reliably transforming *Musa* are now a reality. By working in a network of partners, scientists supported by BIP have exchanged transformation protocols, and verified results. We now know much more about the molecular manipulation of *Musa* including:

- Receptivity of different types of plant materials
- Dependability of DNA introduction methods
- Gene expression
- Time necessary to transform Musa.

As a consequence of transformation technology, BIP-sponsored research has produced banana clones with resistance to BBTV and BBMV that are undergoing glasshouse evaluation and are ready for field testing.

Area 2: Germplasm Collection and Evaluation. Valuable genetic stocks of wild and domesticated Musa species have been placed in accessible collections in India and Vietnam through BIP sponsorship. The future value of these preserved stocks will only be fully realized as scientists evaluate the genetic attributes and combine traits using conventional and molecular tools. Had these stocks not been preserved they would likely have been lost to future generations.

Area 3: Plant Improvement. The projected demise of two critically important banana breeding programs in the Americas was alleviated by the timely awarding of BIP funding. These grants permitted plant breeders to continue to generate improved progeny, and to evaluate genetic selections for industry and subsistence farmers. The practical implication of this outcome is the continuing need for conventional breeding and genetics programs to help find the solutions to production and protection problems faced by banana producers.

Area 4: Pest and Disease Management. BIP's dual strategy for applying genetic transformation and DNA selection marker methods for adding host plant resistance to banana clones for black Sigatoka resistance made significant progress. Transformation technologies (see Area 1) are now available to introduce single gene resistance should suitable candidate genes be found. Secondly, marker assistance selection technologies for multigenic resistance for black Sigatoka appear promising. The value of this strategy is in the expectation that multigenic forms of resistance should be more durable.

Strategies for managing black Sigatoka resistance in *Mycosphaerella fijiensis* proved unreliable in that spray-free periods failed to let the pathogen rebound to fungicide-sensitive forms. This research outcome removes pesticides as a sustainable solution to black Sigatoka, and increases the need for nonpesticide-based integrated pest management (IPM) programs.

Resistance to nematodes is a high priority for bananas inasmuch as chemical controls are costly and hazardous. Identifying sources of resistance, as well as the screening methods developed through BIP-sponsored research, are significant successes.

Impact Assessment

The World Bank commissioned an economic impact study of BIP in late 1998 (see Chapter 4). The key comments and findings are:

1. New knowledge has been produced in the following areas:

- Collection and characterization of banana germplasm that will allow increased effectiveness of the banana breeding programs around the world
- Increased efficiency of breeding programs through improved testing, screening and typing methods, and development of protocols. Additional information gathered through use of genetic markers, particularly for disease resistance to Sigatoka, Panama disease, and nematodes, will also be valuable in future work
- Improved and refined technology for genetic manipulation, including transformation processes for banana and improved knowledge of disease resistance mechanisms in banana
- Use of this knowledge will enhance banana and plantain production, whether for cooking, domestic consumption as dessert bananas, or for export. Applying this knowledge in future to provide real improvements for producers and exporters will require further research and development (R&D) investment.

2. Applied outputs of some of the projects will have immediate value to, and impact on, industry. The following stand out:

- Black Sigatoka-resistant clones further developed in BIP projects are being used to help control an outbreak of the disease in the Amazonas region of Brazil
- Strains of the *Fusarium* wilt pathogen in a given location can now be typed using new

molecular and genetic techniques. This will be particularly beneficial to new banana enterprises in determining where and where not to plant in relation to damaging races of Panama disease.

Although these outcomes are significant, the real value of BIP-supported work will be the long-term strategic knowledge flowing from the BIP projects. Landmark events using some of this new knowledge might be the development of a Cavendish or alternative export banana type that is disease resistant. Other developments that might result include socioeconomic, environmental, and health benefits. The economic benefits would include reduced production costs (fewer chemicals required); the environment would be improved through less chemical usage; and banana producers would suffer fewer health problems by not having to handle as much toxic chemical material. Improvements would lead to better health of workers, higher productivity, and an overall increase in quality of life of communities in banana-producing areas.

The BIP-supported research has been complemented by other significant investments in R&D from public and private sources. These include approximately US\$3 millions per annum invested by the donors to the Consultative Group on International Agricultural Research (CGIAR) for research on subsistance production of banana and plantain by the International Institute for Tropical Agricultural Research in Africa and the International Plant Genetic resources Institute through the International Network for the Improvement of Banana and Plantain.

Leverage of Funds

BIP also leveraged substantial other investments in banana improvement:

 Building on the original CFC investment, further funds have been attracted to BIP, Banana, Breeding, and Biotechnology

within the projects and in complementary activities: for example the Flemish Association for Development Cooperation and Technical Assistance (VVOB) and the Belgium Administration for Development Cooperation (BADC), Belgium, for the nematode consortium personnel positioned around the world in various breeding programs.

- Funding from the New Zealand Government for postharvest practices in Vietnam.
- Funding from the University of Hong Kong and the Governor's Fund in Hawaii.
- Conservative estimate of additional funds contributed is US\$2 million.

Increased Collaboration

A longer-term benefit to industry will be the enhanced scientific cooperation in banana improvement work as a result of the creation of a Biotechnology Consortium (Katholeike Universiteit Leuven (KUL), Belgium, Boyce Thompson Institute (BTI), USA, and Queensland University of Technology (QUT), Australia). This grouping resulted in staff exchanges and the transfer and refinement of particle bombardment and Agrobacterium transformation and regeneration technology. In a similar way BIP fostered the creation of a "nematode consortium," which has resulted in improved screening techniques leading to a better understanding of nematode resistance. Examples of new or enhanced collaboration are:

- Formation of a consortium in biotechnology enabling the refinement of both particle bombardment and Agrobacterium transformation techniques
- Formation of a nematode consortium strengthened during the Project with funding from VVOB and BADC that will continue past the completion of BIP

- Collaboration between Australian and Vietnamese scientists, exemplified in the training of Vietnamese scientists by institutes in Australia
- Collaboration between Australian and CIRAD scientists in screening for Fusarium wilt on CIRAD breeding diploids
- Collaboration between Costa Rica and Brazil in screening breeding materials
- Attraction of new research providers into banana improvement
- Additional permanent staff position created in CIRAD focused on banana improvement
- Continued generic resources provided by Belgium through nematode consortium.

The impact assessment study indicated that BIP should probably provide a rate of return on investment of at least 20 to 33 percent. Conservative assumptions were used in the study, and additional analyses showed robustness of the results. This demonstrates that further investments in banana improvement research will be well justified.

Conclusion

The BIP results have provided methods for transformation and regeneration of bananas, and have moved worldwide banana producers closer to disease- and pest-resistant transgenic bananas. Considerable progress has been made in identifying new germplasm suitable for breeding, and ways have been developed to improve breeding.

BIP has therefore made an important contribution in both the provision of research results,... catalyzing collaborative activities and initiating an internationally competitive research grants scheme. This enabled the inclusion of research providers of excellent quality wordlwide, and the investment of resources in a portfolio of research projects of direct relevance to the objectives of the program. The

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World Bank's overarching commitment is to the alleviation of poverty. The contribution this Project has made toward the development of new banana varieties, and more sustainable methods of disease an pest control in banana and plantain production, is a positive step in reaching this goal.

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2

Banana Improvement Project

B IP was conceived and specially structured to make a significant contribution toward the improvement and productivity of banana, by using higher-yielding, disease-resistant varieties, and seeking ways to reduce the cost of production, especially the cost of pesticide application. A two-pronged approach was taken, based on commissioning research concerned with:

- Genetic improvement, by collecting, developing, and evaluating new banana varieties with export potential, incorporating higher yields and durable disease resistance
- Improved and integrated pest management practices to reduce pesticide use.

The intended beneficiaries of the Project were producers of dessert bananas, particularly smallholders, and subsistence farmers growing plantain and banana for their own or local consumption. Other targeted beneficiaries were plantation workers, because of the focus on improved pest management practices, and the public at large, by decreasing environmental degradation through a reduction in pesticide use, and establishment of plantations in soils and regions that are not optimal to support banana production. Consumers worldwide will benefit through access to a selection of banana varieties grown under more environmentally neutral conditions.

Project Structure

Management

The cosponsors and their technical representatives constituted the governing body of the Project, with the FAO/IGB as the Supervisory Body in accordance with CFC normal practices. The FAO convened a project technical advisory committee to assess overall Project progress. The World Bank acted as the Project Executing Agency, handling the daily management of the Project, and convened a Scientific Advisory Panel to assist in the planning, selection, and monitoring of the research activities. The Bank provided twice-yearly progress and annual reports to the CFC and the FAO/IGB on Project activities. Meetings of the cosponsors were held to discuss progress at the time of the Annual Scientific Meeting.

Project Planning and Implementation

In the early 1980s FAO and its Inter-Governmental Group on Bananas identified the need for greater investment in banana research in response to the increasing cost of production and falling returns to growers. More frequent fungicide applications were required because the major disease, black Sigatoka, continued to spread into banana- and plantain-growing areas. At the same time, fungicide-resistant strains were appearing. Consumers were becoming more concerned about environmental degradation resulting from the increased spraying. Nematicides in particular were being more widely used in an effort to control the major nematode pests. New strains of Fusarium wilt were emerging that later completely destroyed new plantations in the tropics and subtropics. New virus diseases were also being recognized, and banana streak virus in particular was threatening the viability of the international exchange of Musa germplasm to breeding programs. At the same time, markets were expanding, and presenting an opportunity to producing countries to increase their export trade. This was of particular importance to the smaller exporting countries, particularly in Africa, the Caribbean and the Asia/Pacific region, which traded banana as their most significant export commodity.

The World Bank supports banana and plantain research through its contributions to the Consultative Group on International Agricultural Research (CGIAR). The Bank had undertaken a review of international support to banana and plantain research by participating in a CGIAR Task Force on Banana and Plantain (CGIAR 1993) whose recommendations were complementary to the plans of FAO. The FAO/ IGB's overriding interest in *Musa* was focused on the banana export crop, but they also shared the interest of the World Bank and the CGIAR in addressing the problems of growers of banana and plantains for subsistence and the importance of both domestic and export production for poverty alleviation.

The banana and plantain export crop accounts for approximately 10 percent of total production, the remainder being a subsistence crop grown widely in African, Caribbean, and Pacific (ACP) countries. The two organizations therefore collaborated in designing and presenting a project proposal to the CFC, with the resultant Project Agreement being signed during International Centers Week of the CGIAR in Washington, D.C., in October 1993.

The BIP Secretariat was located in the Bank within the Agricultural Research and Extension Group (ESDAR). A Technical Manager was appointed with responsibility for Project implementation, supported by a Project Coordinator. Initial activities of the Bank in the first half of 1994 were the preparation and dissemination of a call for proposals, and the identification of members of a Scientific Advisory Panel (SAP). A review of current banana and plantain improvement programs was also commissioned by the World Bank and a report was prepared on the activities of the current banana improvement programs, and opportunities and innovative ideas identified that could be useful to exploit within the new Project (Buddenhagen, 1996).

The Bank consulted with representatives of the private sector and the donor community during the initiation of BIP. Consultative workshops were held at the time of the SAP meetings (June and December 1994). The June meeting confirmed the relevance of the aims of BIP, with black Sigatoka being the major constraint, and concerns about the use of pesticides, involving increasing costs, environmental degradation, and worker health and safety. Discussion also focused on the newly available techniques of biotechnology, with industry indicating that a genetically engineered Cavendish would be of great interest to them. The use of biotechnology was the focus at the second meeting, where the issue of the likelihood of success in using transformation techniques with subsequent regeneration was discussed.

Research Strategy

The consultations lead to the adoption of the following research strategy:

- 1. New banana varieties:
- Biotechnology-based breeding to genetically modify presently cultivated clones (especially Cavendish types) for a few key characteristics such as black Sigatoka resistance, resistance to BBTV, and delayed ripening to extend shelf life
- Conventional breeding, based on the crossing of improved diploids to generate new hybrids
- Novel breeding schemes based on triploid resynthesis, to develop new banana types with export potential, suitable for niche markets.
- 2. Better disease control practices:
- Identification of the variation in the major pathogens causing black Sigatoka disease and *Fusarium* wilt, to underpin conventional and nonconventional breeding
- Epidemiology and ecology of black Sigatoka disease, as a basis for better management of the disease aimed at reducing the frequency of fungicide applications, while maintaining effective control.

The Project used the innovative mechanism of an internationally competitive research grants program to identify research projects. Two calls for proposals resulted in over 100 submissions from leading research institutes worldwide. Scientists from national, regional, and international research institutes were invited to submit proposals, and combined proposals were particularly encouraged. Proposals were received from institutes in both banana importing and exporting countries, and the public and private sector (Persley and George, 1996).

Research Portfolio

Proposals were reviewed by SAP against a set of merit-based criteria, and were selected to form a cohesive and complementary portfolio to work toward Project objectives. A brief outline of individual project results is given in Chapter 3, organized in technically related areas of activity.

A number of institutes will be continuing BIP experiments into 1999 due to the need for materials to mature prior to analysis. Field testing of transgenic plants produced during the five-year Project is under final negotiation to ensure that regulatory procedures are addressed. Further results will therefore become available in 1999.

Research Achievements

The Chair of the Scientific Advisory Panel, Dr David Mackenzie, observed at the Third Scientific Meeting in November 1998 that BIP-funded research projects have made exciting discoveries, contributed considerable useful new knowledge, and established strong collaboration and linkages that will continue into the future.

"Important advances have been made in the understanding of the genetics of resistance to black Sigatoka. We now have a much greater understanding of what we face with the Fusarium threat. Molecular tools to transform bananas have been developed, and key banana breeding programs have been strengthened as an investment in problem solving. Resistance to nematodes is now better understood, and we have in hand the means of controlling some significant virus diseases of banana. BIP has also demonstrated that chemical control strategies aimed at managing resistance to fungicides of black Sigatoka will not work. We also now know that tissue culture apparently predisposes plants to Fusarium and nematodes.

"BIP has fostered an informal but solid network of scientists that should continue for many years. Some areas of research that need to be addressed within any future framework are listed below:

- Field testing of transformed *Musa* materials must begin as soon as possible. The scientists involved are encouraged to make sure that this happens.
- There is a need to address the emerging biosafety issues regarding field testing of the transformed material. This requires immediate attention.
- All of the institutions involved should address the intellectual property rights questions that revolve around the discoveries associated with BIP-sponsored research.
- We need to develop a better understanding of industry resistance to cultivar replacement, because the banana research community needs industry people as partners.
- The *Musa* research community needs to develop a strategy to address consumer acceptance; that is, issues associated with transformed cultivars. Ignoring this issue could doom some otherwise successful research.
- There needs to be an investment in understanding the genetics of the pathogen of black Sigatoka (*M. fijiensis*). This research should parallel studies of the genetics of host resistance and the development of quantitative trait loci (QTL) probes to enable breeders to breed for durable resistance to black Sigatoka.
- There is a need to develop an understanding of how to use antifungal proteins (AFP) as a part of a black Sigatoka resistance strategy. Will they function as single gene resistance mechanisms and therefore represent a genetic system susceptible to being overcome by pathogen race variation?
- There is a need to merge the excellent *Fusarium* research work with molecular approaches to resistance to banana dis-

ease. This applies as well to nematode research.

- Investment is required in biological control strategy research, given the tantalizing results with *Fusarium* and nematodes.
- Maintenance of conventional breeding capacity is crucial, as it will be needed to put together all of the above.

"BIP, through the project researchers, has delivered many research projects, with many significant scientific advances. Important new methods for enabling others to do research have been devised. A community of *Musa* scholars has been formed, which has generated a renewed interest in what can be done in *Musa* improvement that can no longer be ignored. The work has produced an excellent return on investments, and all principal investigators and collaborators are to be commended for their outstanding work.

"Substantial advances have been achieved in Musa research, but we must build on these successes by generating new funds for both medium- and long-term programs. The programs need to focus on the most promising results and pressing issues to maximize benefits of the research, which should be based on a market-driven approach. The diversification of products to provide choices for diversified demand patterns should be available, and should meet the criteria of consumer markets. The need for software and materials from BIP was highlighted as a response to the urgent need in the Latin American and Caribbean area for improving the livelihood of growers, both in cash value and nutritional value."

BIP Management

Research

The 18 projects (Table 2.1) were identified through an internationally competitive research grants scheme. Proposals were reviewed by SAP against a set of merit-based criteria, and were

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Project ID	Type*	Title	Research Provider	Target Activity
PN1	S	Field and laboratory evaluation of diploid bana- nas for their use in breeding schemes	CIRAD-FLHOR, France	Breeding with biotechnology component
PN2	S	Field crosses for understanding the inheritance of black leaf streak resistance in bananas	CIRAD-FLHOR, France	Breeding with biotechnology component
PN3	S+	Collection, characterization and evaluation of Nendran bananas in India	Kerala Agricultural Univer- sity, India	Breeding
PN4	S+	Collection, evaluation and characterization of genetic resources and improvement of banana in Vietnam	Institute of Agricultural Genetics, Vietnam	Breeding
PN5	A+	Banana breeding in Brazil	EMBRAPA, Brazil	Breeding with biotechnology component
PN8	S	Novel genes for fungal resistance and post- harvest quality	BTI, USA	Biotechnology
PN9	S	Development of transgenic bananas with resistance to banana bunchy top and banana mosaic viruses	QUT, Australia	Biotechnology
PN10 PN11	S S	Molecular tool box Genetic engineering of ethylene biosynthesis in bananas	KUL, Belgium Hong Kong University of Science and Technology	Biotechnology Biotechnology
PN12	S	Use of biotechnology to produce transgenic bananas resistant to BBTV infection	University of Hawaii, USA	Biotechnology
PN14	S	Elimination of banana streak badnavirus (BSV) from improved <i>Musa</i> germplasm and related studies on transmission and host plant/virus/vector interactions	IITA, Nigeria	Biotechnology and breeding
PN15	S	Variability and relationships within populations of <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> from its center of origin	QDPI, Australia	Biotechnology and breeding
PN17	A+	Origin and distribution of fungicide-resistant strains of <i>M. fijiensis</i> in banana plantations in Costa Rica	CORBANA, Costa Rica	Pest and dis- ease control methods
PN18	s	Tolerance and resistance of banana to nematodes	CRBP, Cameroon	Breeding
PN19	S	Identification of durable resistance sources in banana and plantain	KUL, Belgium	Breeding with biotechnology component
PN20	S	Identification of durable resistance sources in banana and plantain	FHIA, Honduras	Breeding
PN21	S	Identification of durable resistance sources in banana and plantain	IRTA, Spain	Breeding
PN22	A	Breeding of hybrid <i>Musaceae</i> with resistance to multiple diseases, especially black Sigatoka and Panama disease	FHIA, Honduras	Breeding

Table 2.1 Constituent Projects of BIP

* S = strategic; S+ = strategic with applied component; A = applied; A+ = applied with strategic component. Data from Chudleigh (Chapter 4).

selected to form a cohesive and complementary portfolio to work in parallel toward the project objectives. Projects were monitored by SAP against progress and annual reports, and following acceptance of their recommendations by the Bank, principal investigators were notified of the review outcome. Continuation of financial support was based on acceptance of reports. Annual Scientific Meetings were also held to enable principal investigators to present results of their work.

Annual Meetings

The cosponsors met in Washington, D.C., in March 1995, Belgium in February 1996, and Guadeloupe in March 1997. The Annual Scientific Meetings were beneficial for information exchange between the cosponsors and technical and scientific advisory panels, and also enhanced the monitoring process. The principal investigators presented and discussed their work with their peers. The meetings allowed participants in the two consortia to discuss and plan project activities, and to interact with other collaborators within the BIP portfolio of projects. This helped establish new and productive external collaborative and networking opportunities.

The Fourth Cosponsors and Third Scientific Meetings were held in Douala, Cameroon, on November 5-6, 1998, followed by a field trip to the CRBP facilities in Njombe. The meeting was timed to coincide with the impending completion of project activities. As in previous years, the meeting venue was selected to take advantage of the presence of *Musa* researchers and the development community who were participating in a *ProMusa* Workshop and International Seminar sponsored by INIBAP, CIRAD, and CRBP immediately following the BIP meetings.

Scientific Advisory Panel

The World Bank convened a Scientific Advisory

Panel to assist in the planning, selection, and monitoring of research activities. The Panel met first in June, 1994 and for the last time on August 4-5, 1998, when the research portfolio was reviewed, and methodology for the impact assessment study was approved. SAP originally had six members, with the Program Manager serving as scientific secretary. Their breadth of experience and expertise covered a wide range of scientific disciplines and knowledge of the banana industry. Following the portfolio selection phase of BIP, the original panel was reconstituted to comprise three members, with additional technical expertise being provided by observers from the CFC and FAO/IGB, and a representative of INIBAP. The members of the Panel and observers over the course of the Project are listed in Appendix 1.

Public/Private Sector Collaboration

The World Bank established an ongoing dialogue with independent producers and multinational companies in the export banana industry from the start of BIP. The purpose was to obtain input from the industry constraints to sustainable banana production, and industry R&D needs. The potential applications of biotechnology to solve the major disease problems were also discussed.

The World Bank is continuing this dialogue with banana producers and commercial companies active in the banana industry as it considers the feasibility of organizing a consortium (*BioBanana*) amongst interested public and private investors to develop new technologies to benefit banana and plantain producers. This concept is described further in Chapter 5.

Impact Assessment

An assessment of the impact of BIP has been done, and the report is included here as Chapter 4. Some of the key comments and findings from that report are:

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Strategic Knowledge

- Increased efficiency of breeding programs through improved testing, screening, and typing methods, and development of protocols; additional information on genetic markers for resistance to nematodes/ *Fusarium* wilt/black and yellow Sigatoka
- Identification of resistance genes for BBTV and BBMV
- Improved and refined technology for genetic manipulation, including transformation systems (Agrobacterium-mediated and particle bombardment)
- Greater understanding of banana streak badnavirus (BSV) vectors and etiology.

Applied Outputs Beneficial to Producers in the Short Term

- Transgenic Cavendish and Bluggoe plants going into glasshouse and field trials
- Support to breeding programs that is enabling EMBRAPA to refine a clone with black Sigatoka resistance, currently being planted in the Amazonas (NW) region; continuation of work at the Fundacion Hondurena de Investigacion Agricola (FHIA), Honduras, on disease-resistant, shortstatured varieties
- Evaluation of Indian Nendran varieties has identified more desirable types for Southern Indian conditions
- Ability to type strains of *Fusarium* wilt in given locations
- Information regarding the continued resistance to chemicals once fungicides are no longer applied.

Leverage of Funds

 CFC investment attracted to BIP, within the projects, and in complementary activities; for example VVOB and BADC, Belgium, for the nematode consortium personnel positioned around the world in various breeding programs

- Funding from the New Zealand Government for postharvest practices in Vietnam
- Funding from the University of Hong Kong and the Governor's Fund in Hawaii
- Estimate of funds contributed is US\$2 million.

Increased Collaboration

- Formation of consortium in biotechnology enabling the refinement of both particle bombardment and Agrobacterium transformation techniques
- Formation of nematode consortium strengthened during the project with funding from VVOB and BADC that will continue past the completion of BIP
- Collaboration between Australian and Vietnamese scientists, exemplified in the training of Vietnamese scientists by institutes in Australia
- Collaboration between Australian and CIRAD scientists in screening for *Fusarium* wilt on CIRAD breeding diploids
- Collaboration between Costa Rica and Brazil in screening breeding materials
- Attraction of new research providers into banana improvement
- Additional permanent staff position created in CIRAD focused on banana improvement
- Continued generic resources provided by Belgium through nematode consortium.

Although these outcomes are significant, the real value of BIP-supported work will be the long-term strategic knowledge flowing from the BIP projects. Landmark events using some of this new knowledge might be the development of a Cavendish or alternative export banana type that is disease resistant. Other developments that might result at least in part from BIP research include socioeconomic, environmental, and health benefits. The economic benefits would include reduced production costs (few chemicals required); the environment would be improved through less chemical usage; and banana producers would suffer fewer health problems by not having to handle as much toxic chemical material (organ damage to an estimated 10 percent of workers according to Swennen 1997). Improvements would lead to better health of workers, higher productivity, and an overall increase in quality of life of communities in banana-producing areas.

A longer-term benefit to industry will be the enhanced collaboration in banana improvement work as a result of the creation of a Biotechnology Consortium (KUL, BTI, and QUT). This grouping resulted in staff exchanges and the transfer and refinement of particle bombardment and Agrobacterium transformation and regeneration technology. In a similar way BIP fostered the creation of a "nematode consortium," which has resulted in improved screening techniques leading to a better understanding of nematode resistance.

The impact assessment study indicated that BIP should probably provide a rate of return on investment of at least 20 to 33 percent. Conservative assumptions were used in the study, and additional analyses showed robustness of the results. This demonstrates that further investments in banana improvement research will be well justified.

Conclusion

BIP results have provided methods for transformation and regeneration of banana, and have moved worldwide banana producers closer to the reality of a disease- and pest-resistant transgenic banana. Considerable progress has been made in identifying new germplasm suitable for breeding, and ways have been developed to improve the breeding programs. The independent impact assessment clearly indicates that the most important output from BIP is strategic new knowledge that can be used for future research and development projects.

BIP has therefore made an important contribution in both the provision of research results, catalyzing collaborative activities and initiating an internationally competitive research grants program. This enabled the identification of research providers of excellent quality worldwide, and investing resources in a portfolio of research projects of direct relevance to the objectives of the program.

The World Bank's overarching commitment is to the alleviation of poverty. The contribution this Project has made toward the development of new banana varieties, and more sustainable methods of disease an pest control in banana and plantain production, is a positive step in reaching this goal.

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Project Reports

The 18 BIP-funded projects are described in this chapter, in a format that will allow easy comparison, and ease of use in other areas. The projects have been grouped into four broad areas as follows:

Area 1 – Biotechnology

- Area 2 Germplasm Collection and Evaluation
- Area 3-Plant Improvement
- Area 4-Pest and Disease Management

The information has been extracted from project completion reports, the BIP impact assessment study, and file materials. Readers requiring more detailed information are encouraged to contact the principal investigators.

Area 1 – Biotechnology

PN8: Biotechnology Consortium: Novel genes for fungal resistance and postharvest quality

Boyce Thompson Institute for Plant Research, USA

Principal investigators: Charles Arntzen and Greg May

Activity. The researchers sought to create a "molecular toolbox" in collaboration with PN9 and PN10. The toolbox is for use in isolating and manipulating new genetic elements from *Musa* spp. The toolbox will include Grande Naine genomic libraries, cDNA libraries representing mRNAs present in a variety of banana tissues at different developmental stages or under pathogen attack, and a variety of *Agrobacterium* strains for *Musa* transformation. Work at BTI was related to genes associated with fruit ripening and fungal resistance.

Background. Development of the toolbox will allow the creation of new banana germplasm with resistance to virus and fungal diseases that now limit production and increase production costs. It will also provide a better fundamental understanding of the mechanisms controlling qualitative and quantitative changes in gene expression that occur during banana fruit development or under pathogen attack.

Results. Identification and characterization of eleven distinct classes of differentially ex-

pressed cDNAs from the pulp of ripening Grande Naine banana fruit. Efforts continue toward the isolation of regulatory elements governing the expression of mRNAs.

Transformation cassettes incorporating these regulatory elements were built.

Identification of previously uncharacterized mRNAs from banana roots and leaves.

Development of a transient assay system based on protoplasts derived from readily available banana pulp.

cDNA libraries were generated from leaves of Gros Michel, Malaccensis, and a Cavendish variety that were challenged by *Mycosphaerella fijiensis*.

Rapidly doubling Grande Naine embryonic cell suspensions have been developed.

Outcome. This project was completed in February 1997. Since then further work has been carried out in the various areas above. Cloned genes have been distributed to other laboratories both within the consortium and to the wider research community. Further refinement of the Agrobacterium-mediated transformation system has been undertaken by identification of alternative sources of starting materials, the aim being to reduce the number of chimeric plants obtained. Several banana-derived promoter elements are being isolated and characterized that will allow for the tissue- or developmental-specific expression of foreign genes in transgenic banana plants. The project also gained a better understanding of the molecular events that occur during banana fruit development and ripening, and those events that are associated with pathogen challenge in fungal-resistant and nonresistant banana varieties. The molecular toolbox has provided many laboratories with molecular biology materials.

Impact. The major impact of this project has been the additional knowledge generated on ethylene control, and the further development of the *Agrobacterium* method of transformation. The project contributed to strengthening the collaborative activities of the BTI group as a member of the biotechnology consortium, with visiting scientists, and through sharing of technologies.

PN9: Biotechnology Consortium: Development of transgenic bananas with resistance to banana bunchy top virus and banana bract mosaic virus

Queensland University of Technology, Australia Principal investigator: James Dale

Activity. In collaboration with other members of the Biotechnology Consortium (KUL, Leuven; BTI, USA); and with the University of Hawaii (USA), the scientists attempted to control BBTV and BBMV through the development of multivirus-resistant transgenic bananas. The team utilized current and emerging knowledge of BBTV resistance, and characterization of the BBMV genome, to develop transformation cassettes. Transformation proceeded initially on single resistance transgenes, and ultimately to multiple resistance transgenes. Transformed material was screened for resistance, and promising transgenic lines prepared for field trials.

Background. BBTV is largely controlled in Australia by removal of infected plants, a costly undertaking. Cavendish is particularly susceptible to the disease, and can reduce yields by up to 100 percent, but averages at about 30 percent. BBMV creates losses of between 20 and 40 percent. A range of cassettes has been generated that potentially confer resistance to BBTV and BBMV in transgenic bananas, and have been used in the transformation and regeneration of Bluggoe and Cavendish embryos. Three different BBTV, and one BBMV, resistance cassettes were used, resulting in regenerated plantlets that are ready for virus challenge in the field. A range of promoters for transgene expression continues to be analyzed for use in generating virus resistance.

Results. Identification of potentially three different BBTV resistance genes, and one BBMV

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gene. Cavendish and Goldfinger plants have been regenerated from embryonic cell suspensions, using the particle bombardment transformation method.

- Cavendish "Grande Naine" embryos have been transformed and regenerated with GUS, GFP, two different BBTV resistance cassettes, and one BBMV resistance cassette.
- Additional embryonic cell suspensions have been generated, and new virus resistance constructs have been designed that are expected to be more efficient in generating resistance.
- Planning for initial greenhouse evaluation and field trials is progressing to ensure that biosafety regulations are addressed, and to minimize the possibility of loss of material.
- Three unexpected problems arose during the project that have delayed the realization of final results: Cavendish transformation and regeneration is slower and less efficient than Bluggoe; the erratic rate of multiplication of transgenic lines for virus challenge, and identification of a suitable screening site for BBMV proved more difficult than expected. These problems have now been overcome.

Outcome. The technology developed under the project is directly applicable to the generation of transgenic resistance to other diseases. The development of an efficient transformation and regeneration protocol for Cavendish, and identification of promoters to drive high level transgene expression, can be utilized together to express novel genes in banana. It is expected that of the current transgenic lines produced, 50 percent will test resistant, which will increase as the technique is further refined.

Impact. Technology to generate potentially virus-resistant *Musa* varieties, including transformation, regeneration, and expression of new genes, has been demonstrated. Confirmation of

resistance has yet to be tested in the field, and it is expected that resistance breakdown will be about 5 to 10 percent within a period of 10 years of release of resistant types. Technologies developed are relevant not only to viral resistance, but also to other diseases of banana and plantain types. Access to BIP influenced the QUT approach, thereby playing a positive role in the successful results.

PN10: Biotechnology Consortium: Genetic transformation of prototype bananas for black Sigatoka and *Fusarium* resistance

Katholeike Universiteit, Leuven, Belgium Principal investigators: Rony Swennen and Laszlo Sagi

Activity. In collaboration with PN8 and PN9, the main focus of the project was the investigation and optimization of a reliable and transferable transformation system. Agrobacteriummediated transformation and particle bombardment techniques were tested for their suitability and efficiency for large-scale applications. Other project activities were the isolation of a strong banana promoter, high molecular weight DNA for large insert genomic libraries, the preparation of a leaf cDNA library, and the construction of expression vectors with selectable marker genes for banana transformation.

Background. The impetus for this project came with the significant progress that had been made in in vitro manipulation of banana in the early 1990s. Highly regenerable embryonic cell suspensions (ECSs) were established at KUL from vegetative meristematic tissue. The material enabled the demonstration, for the first time, of banana transformation at the level of transient gene expression in electroporated protoplasts. At the same time regenerable ECSs also showed a great potential for direct generation of transgenic banana plants by particle bombardment. This BIP project therefore built on the previous work to develop or adapt these new biotechnological tools for genetic manipulation of banana and plantain, and apply them to genetic improvement.

Results

- Particle bombardment of embryonic cell suspensions proven to be the superior transformation method
- Plant expression vectors containing chimeric selectable marker genes for the two best selection systems of banana cells were constructed
- Several heterologous promoters driving high gene expression in banana were identified
- New assays and bacterial test strains were developed to allow for the study of interaction between banana and *Agrobacterium tumefaciens*
- A cDNA library was prepared from banana leaves for the isolation and characterization of specifically expressed genes
- Partial isolation and characterization of an actin gene from a Grande Naine genomic library was carried out.

A protocol was described for the isolation of high molecular weight DNA banana leaf nuclei, resulting in library-quality DNA for the preparation of large insert genomic libraries.

Outcome. The particle bombardment method was used by collaborative projects within BIP, and made available for molecular breeding of banana in independent laboratories worldwide. Optimal conditions are now defined for the most critical steps of a technology for particle bombardment of embryonic banana and plantain cell suspensions. This has many practical applications, the paramount one being the production of material resistant to major pests and diseases. The *Agrobacterium*mediated transformation system needs significant improvement, although work on this activity has supplied some useful information. New transformation vectors have been designed that are useful for sequential transformations to introduce multiple genes, and also provide a readily available backbone to insert any potentially interesting selectable marker gene for testing in banana. These vectors enable more versatile and routine applications for molecular breeding of banana.

Impact. BIP accelerated the ability to transform banana by refining the current systems, and widening the parameters of exploration. BIP assisted in knowledge generation of the techniques enabling other laboratories to use the technologies. This BIP project was the catalyst for the collaborative link between the participating laboratories, which extended to the University of Hawaii (PN12). Through this project, and others in the biotechnology consortium, BIP has played a role in the acceptance of potential biotechnology solutions to the major problems of banana production by the private sector.

PN11: Genetic engineering of ethylene biosynthesis in bananas

Hong Kong University of Science & Technology, Hong Kong Principal investigators: Shang-Fa Yan and Ning Li

Activity. The aim of this project was to clone genes involved in ethylene biosynthesis in banana, the genetic transformation of banana with reduced ethylene production capacity, and the evaluation of transformed plants in relation to reduced disease development and fruit quality. Due to time constraints on the project, only the first objective was addressed.

Background. The plant hormone ethylene is produced by the banana plant and fruit, and plays an important role in disease susceptibility and fruit ripening. Ethylene production is induced by pathogen attacks resulting in accelerated leaf senescence and yellowing, and can also cause premature ripening of the fruit resulting in reduced storage life and poor quality. It has been established that 1-aminosyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase genes participate in the regulation of, and catalyze the last two steps in, the pathway of ethylene biosynthesis. Although these genes have been cloned in a number of other species, cloning in banana had not been achieved. The recent advances in genetic engineering of higher plants offered the possibility of using biotechnology to genetically engineer crop plants with endogenous ethylene production in fruits, resulting in prolonged storage life.

Results

- Construction of a banana genomic library and identification of some ACC synthase (ACS) and ACC oxidase (ACO) clones
- Characterization of the clones by restriction enzyme digestion and partial DNA sequencing
- Complete sequencing of one ACS and two ACO genomic DNA clones
- Elucidation of the regulation of ACS and ACO gene expression by developmental and environmental factors by performing an RNA gel blot analysis
- Delivery of an antisense ACS and ACO gene construct to KUL for investigation.

Outcome. This project has generated substantial new knowledge on the issue of ethylene production, and highlighted the potential that, with further investment, postharvest losses during transportation and storage can be significantly reduced. KUL researchers advised that genetic transformation was successfully performed on the gene construct, but a mite problem on the resultant material has delayed the work. A rehabilitation program is now underway. Further support is required for this activity to build on the results in linking the gene structure to their functions in disease resistance and fruit ripening.

Impact. The increased knowledge of the genetic information associated with ethylene bio-

synthesis will be useful in future research aimed at postharvest qualities of fruit, as well as disease control. The study has laid the foundation for further analysis of the fruit ripening specific promoter, and provided the basis for genetic engineering of ethylene biosynthesis and metabolic pathways in banana.

PN12: Use of biotechnology to produce transgenic banana resistant to banana bunchy top virus infection

University of Hawaii, U.S.A. Principal investigator: John S. Hu

Activity. The objective of this project was to produce transgenic banana resistance to BBTV by introducing viral genes into the genome. The project utilized and modified both systems developed in the "molecular toolbox" of the consortium, particle bombardment or "gene gun" technology, and the Agrobacterium methods. Although not a member of the Biotechnology Consortium, the University of Hawaii group has worked closely with its members.

Background. BBTV is the most devastating virus disease of bananas in many producing areas, including Asia, Africa, and the South Pacific. Since 1989 the Hawaii Department of Agriculture has been attempting to eradicate the virus, an approach that worked well in Australia. This approach appears insufficient in Hawaii, indicating that additional control strategies and long-term solutions to the problem must be found. Development of resistant varieties based on breeding efforts has been complicated by ploidy level differences and sterility in the commercial clones. Recent developments in biotechnology offered a new avenue.

Results

• Two banana transformation and regeneration systems were evaluated for development of transgenic banana plants.

- Several transformation experiments were conducted with various BBTV-gene constructs.
- Transformed Cavendish Williams bananas containing the BBTV gene constructs were obtained, using a modified version of the *Agrobacterium*-mediated transformation system developed by the BTI group.
- Transgenic banana plants were screened in the greenhouse, resulting in the identification of BBTV-resistant material.
- Evaluation in field trials is planned, however the BIP-funded research is now completed, and funds are required to undertake field experiments.

Outcome. This project has provided evidence that pathogen-derived resistance works for BBTV-like viruses, and with further support varieties can be developed for banana growers in Asia, Africa and the South Pacific, where BBTV is a major problem at production levels. The modified Agrobacterium system will also be useful for other researchers working on resistance to other diseases, including black Sigatoka. Sixty-five transgenic plants have been evaluated in the greenhouse for BBTV resistance, and of these, three plants did not develop any BBTV-specific symptoms, and registered negative in ELISA tests. The project has also succeeded in refining a protocol to identify transgenic cells. Gene constructs from this project have been made available to KUL and the Centro Agronomico Tropical de Investigacion y Ensananza (CATIE) in Costa Rica, and are available to other researchers on request.

Impact. Field experiments had not been conducted when BIP ended to challenge the transformed plants against BBTV. Further work also needs to be undertaken using BBTV virus groups from other areas where the disease occurs. Current results, however, are highly significant in that it has been demonstrated that viral-resistant plants can be produced, and that further refinement of the technique could translate into considerable reduction in the losses caused by the virus in Asia, Africa, and South Pacific countries.

Area 2—Germplasm Collection and Evaluation

PN3: Developing improved banana varieties with pest and disease resistance, postharvest superiority and maximum export potential Kerala Agricultural University, India Principal investigator: K. Aravindakshan and Rema Menon

Activity. The collection, characterization, and evaluation of Nendran banana in India undertaken in close collaboration with the national banana program (NRCB), was intended to establish reference data for Nendran for use by future researchers. Selected clones from the collection were evaluated for dwarfness, short cycling, and disease resistance, particularly for yellow Sigatoka, and to a lesser extent weevils and nematodes.

Background. The project concentrated on collection and characterization of the Nendran type AAB plantain banana and wild relatives. Nendran varieties are the most important type grown in the South Indian peninsula, and are exclusively cultivated on commercial lines. They have not, however, been systematically evaluated for optimal breeding characteristics. Characterization of the Nendran-type germplasm is based on morphological and isozyme markers.

Results

- One hundred and twenty-five accessions available for characterization; 111 Nendran, and 3 wild types were collected from 8 locations. Fourteen accessions from the germplasm bank at the Banana Research Station, Kannara, were included in the study.
- The collection was established in the field at the Banana Research Station, Kannara,

and the passport data for each of the collections have been prepared.

- Morphological characterization was carried out using the INIBAP descriptors. Differences have been observed in color, height, and aspect of pseudostem, inflorescence, and bunch characters.
- Tentative identification of 14 types has been made, and a key developed.
- A database has been established containing information on the collection.
- Procedures for isozyme polymorphism studies have been standardized, with five enzyme systems surveyed in various accessions. Results indicated a variation in the banding pattern as well as intensity of individual bands.
- Reaction of accessions to yellow Sigatoka was studied and reported, with further trials underway.
- Reaction of accessions to nematode attack was assessed and reported.

Outcome. Variations in Nendran types collected are being documented on a reference database. This will enable identification of varieties with superior agronomic traits, such as tolerance to drought and water logging, which is of particular interest in Southern India. Accessions collected and established at Kannara will be available for future collaborative programs.

Impact. Southern Indian breeding programs are now able to select material better suited to the various farming systems. The possibility of identifying a short-statured variety has been increased, which will assist in lowering production costs since propping would not be required. Germplasm has been collected that could be useful for breeding programs worldwide.

PN4: Collection, evaluation, and characterization of genetic resources and improved banana crop plants in Vietnam

Institute for Agricultural Genetics, Hanoi, Vietnam

Principal investigator: Do Nang Vinh

Activity. The major focus of this project was the collection of banana germplasm, and its evaluation for productivity and disease resistance to enable selection of varieties with high export quality. The project also focused on micropropagation techniques for elimination of virus diseases. Cultivars displaying desirable agronomic and fruit qualities are being selected for use in the Vietnamese breeding program.

Background. Located in the center of origin of banana, the Vietnamese crop is highly diversified with many low-yielding and disease-susceptible cultivars being grown, resulting in a low annual yield of approximately 15 tons/hectare, and below standard quality of export fruit. Even so, banana is reported as being the most important fruit crop in Vietnam, contributing significantly to the income of millions of farm households. Studies have shown that genotypes in the collection are not represented in existing collections anywhere in the world. This presents a valuable source of parent material for breeding programs. The collection has been established in vitro. The project was strengthened through training visits by selected scientists to laboratories in Queensland, Australia, to learn techniques of identification of banana diseases, and banana production technology. A further positive activity was the convening of an international seminar in 1995 that attracted the participation of a wide range of Vietnamese officials. This culminated in the formation of an internal banana research network, with close contacts between Institute for Agricultural Genetics and the Fruit Research Centre in Phu Ho, Vinh Phu, for banana genetic resource studies.

Results

- A large number of banana cultivars, clones, and wild species were collected, preserved, and characterized.
- Material displaying the most important agrobiological characters was released for production. Guidelines for field manage-

ment based on individual agrobiological traits of each cultivar were recommended to farmers in an effort to achieve the highest economic returns.

- Improved banana micropropagation technologies were achieved, aimed at reducing production costs of micropropagated plants. The technology and disease-free materials were transferred to a number of production laboratories in Vietnam, enabling them to supply large quantities of high-quality microplants for production.
- Information gained on levels of disease susceptibility and pathogens of banana genotypes will enable the study of biocontrol and genetic improvement of disease resistance in banana.
- The addition of genetic resources from introduced material, and the addition of tetraploids to the Vietnamese collection by in vitro polyploidation, is providing valuable new materials for future banana breeding programs.

Outcome. Higher economic returns for farmers are expected from improved understanding of production practices. Genetic resource activities are providing information to enable selection of potentially useful cultivars for breeding programs both in Vietnam and internationally. Many of the accessions being analyzed have not previously been collected, therefore this project is providing a valuable source of material with potential for disease and pest resistance and quality characteristics. The project provided the impetus required in Vietnam to focus on this important commodity, including the training of scientists.

Impact. The project has allowed scientists in Vietnam to address issues of banana research for the first time. New material has been collected that is potentially useful for worldwide breeding programs. An internal network has been established with other *Musa* research institutes, and strong linkages have been made with Australia as a result of a scientific training course arranged with PN 15 at the Queensland Department of Primary Industries.

Area 3–Plant Improvement

PN 1: Field and laboratory evaluation of diploid bananas for use in breeding Centre de coopération internationale en recherche agronomique pour le développement (CIRAD/FLHOR), Guadeloupe Principal investigator: Christophe Jenny

Activity. The objective of this project is to supply breeding programs with reliable information on diploid lines with desirable characteristics for use as parent stock. Agronomic and laboratory characterization methods are being used, as are wild and cultivated clones.

Background. Collaborative arrangements for evaluation were undertaken between CIRAD-FLHOR Guadeloupe, Montpellier, and New Caledonia; WIBDECO, Saint Lucia; and the Queensland Department of Primary Industries (QDPI, PN15), Australia. Evaluations focused on agronomic and morphological traits, molecular characterization, and resistance to black and yellow Sigatoka, and *Fusarium* wilt Race 4. Accessions were obtained from the CIRAD-FLHOR collection and the INIBAP Transit Center.

Results

- Indexed and virus-free accessions were multiplied and sent to prepared field sites for disease resistance evaluation. Evaluation protocols were devised for New Caledonia (black Sigatoka resistance trials) and Saint Lucia (yellow Sigatoka resistance trials). Accessions were screened for *Fusarium* wilt disease in Australia at QDPI. Accessions were tested for their agronomic expression in Guadeloupe due to the absence of most major diseases.
- Isozyme, RFLP, and microsatellite analyses were performed on all existing accessions

in Guadeloupe, and morphotaxonomic descriptions done. Complete characterization of the diploid clones is being undertaken using all available markers.

- Accessions new to the Guadeloupe collection were assessed for their ploidy level by flow cytometry methods, and are currently being evaluated using all available markers. This work will result in the classification of clones by genotypic and phenotypic characters, and will assist in the identification of relations between wild and cultivated diploids.
- Indexation results are being awaited from Australia to enable the final batch of accessions to be dispatched for *Fusarium* wilt and yellow Sigatoka screening trials.

Outcome. This project is providing helpful information on diploid material used by all breeding institutes, and is a valuable addition to the existing information on banana accessions held in genebanks around the world. This rational basis is important to identify clearly the diploid material of the greatest potential within the available germplasm, natural or not, in order to optimize the use of resources available to breeding programs.

Impact. Information has been supplied on diploid materials that is useful within all breeding programs. This has been of immediate use in the CIRAD and CRBP programs, and is also available to others. It is expected that use of this information will result in a shorter timeframe to produce suitable hybrids with the required traits. CIRAD has conservatively estimated that this new knowledge will result in a ten-fold increase in the number of dessert bananas produced.

PN2: Field cross experiments for the purpose of understanding the mechanism governing the inheritance of Black Leaf Streak resistant characters in banana

Centre de coopération internationale en recherche agronomique pour le dévelop-

pement (CIRAD-FLHOR), Guadeloupe/ Montpellier Principal investigator: Frederic Bakry

Activity. All breeding programs are seeking the development of varieties showing resistance to black leaf streak disease (BLSD) to lessen the use of fungicides, the cost of production, environmental damage, and human health concerns. Field resistance screenings against BLSD led to the identification of two forms of resistance reported as highly resistant (HR) and partially resistant (PR). Within the parameters of this project, experimental crosses were undertaken with three wild Musa acuminata accessions expressing the HR, PR, and susceptible (S) reactions against BLSD. Moreover, the project aimed at establishing linkages between field observations and molecular studies in order to identify quantitative trait loci (QTL), and to localize the main resistance genes on a saturated genetic map of bananas.

Background. Ongoing field test screening for resistance indicates that the environmental effect on gene expression is significant, and that controlled environment testing is necessary for a precise analysis of the inheritance of the HR and PR resistance forms. Therefore, research on the PR form of resistance was dropped because it was found that field evaluation procedures routinely used were not precise enough in this case for genetic analysis. The project focus on observation of resistance pattern of hybrids arose from crosses involving the HR resistant parent, and their correlation with molecular data for the localization of the main resistance genes.

Results

• The phenotype of most of the 153 clones of the F₂ progeny from the HR x S cross has been confirmed. Questions remain about the phenotype of some hybrids (HR versus highly PR). This was undertaken by field evaluation

on at least two cycles in two locations, Njombe and Ekona, CRBP, Cameroon.

- Molecular characterization of the F₂ progeny has been undertaken in CIRAD where the genotype has been characterized for 39 polymorphic codominant markers (RFLP + microsatellites) and 110 polymorphic dominant AFLP markers. Results showed significant allelic distorted segregations (for 58 markers) in the F_2 progeny that highlight the use of molecular study for the inheritance analysis. A first mapping was concluded to join the markers in 11 linkage groups. Correlation made with field observations ranked in six classes of resistance, and a second RFLP marker mapped on a different linkage group with a lower significance level.
- Observations made on the F₁ progeny from the HR x PR cross concluded unequivocally that the PR phenotype is dominant over the HR phenotype in bananas.

Outcome. For a precise identification of resistance genes, protocols for glasshouse trials are being developed by CIRAD. This activity is designed first to confirm in controlled conditions the resistance phenotype of the hybrids, and second to identify all different genes involved in the resistance, even minor genes, for a durable resistance. The main genes/QTLs involved have been on the genetic molecular map. This will enable the early screening for BLSD resistance plants or hybrids to be done directly on in vitro plants with molecular markers (marker assisted selection). The banana genetic molecular map first developed by CIRAD is being completed, and many codominant markers have been mapped and information is available for any gene/QTLs location and identification. This work contributes to the breeding of BLS-resistant plants that are economical and ecological necessities.

Impact. Molecular marker assistance in the identification of resistance genes for black

Sigatoka will initially be useful for conferring partial resistance to black Sigatoka through breeding programs. The assumption is that this will decrease the spraying frequency for black Sigatoka by 50 percent or more. The understanding and identification of genetic resistance mechanisms will be a critical component of future strategies to reduce the economic and environmental impact of existing chemical control methods.

PN5: Banana breeding in Brazil Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil Principal investigator: Sebastiao de Oliveira e Silva

Activity. The aim of this project was to breed high-yielding varieties of banana resistant or tolerant to the main pests and diseases, with agronomic qualities of low plant height, short production cycle, and optimal fruit bunch and fruit size. This was attempted by the production of superior diploid and tetraploid cultivars and identification of new varieties; and the development of techniques for in vitro fertilization and for propagation with reduced somaclonal variation. With the arrival of black Sigatoka into the Amazon region in 1998, the objectives of this project became even more critical than previously.

Background. Banana production is an important agricultural activity in Brazil, with high economic and social benefits. The cultivated area covers about 516,000 hectares. The industry is based on smallholder farmers, and is an important source of employment. BIP enabled the Brazilian program to be recognized internally and internationally. Valuable diploids were evaluated and selected as breeding parents to create diploid and tetraploid cultivars, as well as AAB dessert cultivars. This material was evaluated for resistance to rhizome weevil borer, *Fusarium* wilt, Moko disease (Bacterial Wilt), and yellow and black Sigatoka. In tests to evaluate for nematode and insect resistance, researchers have found one hybrid that is tolerant to the rhizome weevil (*Cosmopolites sordidus*), and two hybrids that are tolerant to the nematode *Radopholus similis*.

Results

- Diploid hybrids are being obtained by introducing preselected genotypes and/or new material from other breeding programs.
- Diploid and tetraploid hybrids were evaluated for resistance to black and yellow Sigatoka, *Fusarium* wilt, and nematodes, as well as agronomic characteristics and fruit quality.
- The resultant hybrids were evaluated for 23 desirable characteristics. Twenty-eight diploid hybrids, and 13 tetraploids (12 Prata and one Maca type) were selected.
- The tetraploid (AAAB) hybrid PV-03-44 and the cultivar Caipira, were recommended for commercial planting in the Amazon region.
- Techniques have been refined for in vitro fertilization, somatic embryogenesis, micropropagation, somaclonal variation, molecular characterization of germplasm, and somatic hybridization.
- Generation of AAAB seeds and seedlings from crosses of Maca AAB and AA diploid PA Mysore 2.
- Development of a device to measure the resistance to fruit detachment for use during germplasm evaluation.

Outcome. The arrival of black Sigatoka into Northern and North West Brazil made this project one of considerable urgency. Two new varieties, a cultivar *Caipira* and hybrid PV03-44, partly developed under BIP and resistant to black Sigatoka, are being used as a barrier in an attempt to avoid the spread of the disease to the plantation growing areas in Southern Brazil. These and other materials that are being developed under BIP will provide valuable breeding lines for international breeding programs. Viable seeds and seedlings of AAAB types are considered an important result of the project, since no Maca type tetraploid hybrid had been generated previously.

Impact. The project resulted in improved selection of breeding lines for EMBRAPA programs for resistance to black Sigatoka, and other pests and diseases of banana and plantain. BIP enabled the Brazilian breeding program to accelerate trials in various ecosystems, assisting in the development of a Sigatoka-resistant clone. This clone is currently being planted in the Amazon as part of a program to prevent further distribution within Brazil of this newly arrived disease, which is potentially devastating for the industry.

PN22: Breeding hybrid Musaceae with resistance to multiple diseases, especially black Sigatoka and Panama disease

Fundacion Hondureña de Investigacion Agricola (FHIA), Honduras Principal investigator: Philip Rowe

Activity. This project builds on the existing breeding program to obtain hybrid bananas with resistance to multiple diseases, including black Sigatoka and Panama disease, by conventional breeding.

Background. Pollinations were made onto established and new plantings of the seed-fertile Lowgate (the shortest dwarf mutant of Gros Michel) and Dwarf Prata triploid parental lines for the production of tetraploid hybrids. The two main diploid pollen parents used in these 3x x 2x cross-pollinations were the burrowing nematode-resistant SH-3142 and the race 4 of Panama disease-resistant SH-3362. The SH-3444 tetraploid was also pollinated in 4x x 2x crosses to produce triploid hybrids. It has previously been verified that the 4x x 2x approach is a highly effective way of breeding new cooking bananas. It is therefore anticipated that breeding for a triploid export dessert banana will also be successful.

Results

- Of the 2200 Lowgate bunches harvested, 16 seed were produced and 10 had embryos that were cultured for germination. Four of these 10 embryos were viable, and three of the plants produced have been evaluated in the field. These three mature tetraploid plants with Lowgate parentage had the desirable Grande Naine-type plant height, but were susceptible to black Sigatoka and had mediocre bunches.
- These results illustrate that much higher numbers of Lowgate bunches should be pollinated to produce larger populations from which selections could be made. This would greatly increase the possibility of selecting a hybrid with black Sigatoka tolerance, and the bunch qualities of FHIA-23, but which would also have the desired shorter plant height. FHIA-23, which was derived from Highgate x SH-3362, is tolerant to black Sigatoka and has excellent bunch features.
- From the 450 Dwarf Prata bunches harvested, 685 seeds were produced and 250 had embryos. From these 250 cultured embryos, 123 germinated and most of these hybrid plantlets were transplanted to the field for evaluation.
- The 600 bunches of SH-3444 yielded 84 seeds and 20 cultured embryos. From these 20 cultured embryos 17 germinated. Two of these 17 plants have the gene for dwarfness and only these dwarf plants will be transplanted to the field for further evaluation.

Outcome. The results of this project demonstrated that dwarf tetraploid export bananas could be bred. However, larger segregating populations must be produced to expect subsequent selection of dwarf plants with the desired bunch qualities and resistance to diseases. In view of the proven effectiveness of both the 3x x 2x and 4x x 2x breeding schemes for produc-

ing tetraploid and triploid commercial hybrids, it is critical that these activities be continued.

Impact. The ability of FHIA to produce varieties displaying the desirable traits of pest and disease resistance, short stature, and good fruit quality was assisted by BIP, which will have a major impact on banana production when achieved. Field trials will be continuing on the various crosses undertaken by the project.

Area 4-Pest and Disease Management

PN15: Variability and relationships within populations of *Fusarium oxysporum* f.sp. *cubense* from its center of origin Queensland Department of Primary Industries, Australia Principal investigator: Ken G. Pegg

Activity. This project was intended to provide information on the genetic diversity within populations of *Fusarium oxysporum* f.sp. *cubense* (*Foc*) in various areas in the Asia/Pacific region, and the risk this poses to banana production. The reliability of using tissue culture-derived plants as opposed to conventional planting materials when assessing pathogen resistance was also addressed. The project will enable breeding and selection programs to screen genotypes against a more complete representation of the pathogen. Isolates, lyophilized during the study, will also be available for use in virulence and pathogenicity tests in countries that are able to test nonendemic strains.

Background. There are no control mechanisms for Panama disease, meaning that once infected the plantation has to be abandoned, with the pathogen remaining in the soil for long periods. Studies have been undertaken on genetic diversity of *Fusarium* as well as on field evaluations of potential breeding materials. Specific parts of the ribosomal DNA within the *Fusarium* wilt fungus were characterized and sequenced, and this information was used to identify genetic variation among the different races of the fungus. Associated activities included the planning and execution of a sixmonth training course for four scientists from the BIP-funded project in Vietnam, and collaboration with CIRAD-FLHOR Guadeloupe, in screening diploid breeding materials for *Fusarium* wilt.

Results

- Established a worldwide collection of over 2000 isolates of *Foc* (Africa, South and Southeast Asia, North and South America, Australia) that have been lyophilized and will serve as a reference collection
- Determined diversity of *Foc* through the use of vegetative compatibility groups (VCGs), DNA fingerprinting, and volatile groups
- Determined the global distribution of strains of *Foc*. Conducted in-depth studies on the pathogens from the presumed center of coevolution
- Identified strain-specific PCR primers for development of PCR-based detection systems for *Foc*. Developed a library of DNA banding patterns
- Characterized and sequenced the internal transcribed space region and the intergenic spacer region (IGS) of the ribosomal DNA (rDNA) genes of *Foc*. Identified genetic variation among different races and VCGs based on IGS haplotype. Constructed a phylogeny for the genotypes of *Foc* based on DNA fingerprinting and IGS sequence information
- Developed protocols for field evaluation of hybrids and diploids for resistance to *Fusarium* wilt
- Demonstrated the greater susceptibility of plants derived from tissue culture to *Fusarium* wilt when compared to conventional seed pieces.

Outcome. Information has been provided on the pathogenic and genetic diversity of *Foc* in

various regions, enabling breeding and selection programs to screen Musa genotypes against a more complete representation of the pathogen. The project established that maximum variability was found in the Asia region, considered the center of origin for Foc. Until this study, Fusarium wilt in Cavendish cultivars was thought to be confined to the subtropics, due to cool winter temperatures. Since no predisposing factors have been recognized in Southeast Asia and northern Australia, isolates in VCGs 01213 and 01216 appear to constitute a tropical Race 4 that has the capacity to threaten the world export trades. The only way to control *Fusarium* is through host resistance, therefore disease-resistant germplasm is essential to any breeding program, together with knowledge of the genetic nature of resistance. Consequently CIRAD-FLHOR have sent their breeding diploids to Australia for field evaluation. Work on the identification of soil-borne resistance organisms will enable inoculation regimes to be designed for micropropagated plants prior to field planting.

Impact. The collection of various strains of Foc will enable the more efficient identification of the pathogen, being especially useful in the identification of suitable plantation locations. The increased knowledge on genetic diversity will be useful to breeding programs to screen against a more complete profile of the pathogen, as will the protocols for field evaluation of hybrids and diploid materials. Quarantine or exclusion procedures need to be quickly implemented in the Asia/Pacific region to confine tropical Race 4 populations to its existing areas of distribution. The finding of a Musa acuminata ssp. population segregating for resistance to subtropical Race 4 should be useful in developing molecular markers, leading to the identification of host resistance genes. BIP has developed and strengthened professional links between the project and the world's major breeding programs, particularly FHIA, CIRAD, and EMBRAPA.

PN17: Origin and distribution of fungicideresistant strains of *M. fijiensis* in banana plantations in Costa Rica

Corporación Bananera Nacional (CORBANA), Costa Rica Principal investigator: Ronald Romero-Calderon

Activity. This project sought to increase knowledge on the dynamics of isolates of *M*. *fijiensis* (the causal agent of black Sigatoka) resistant to fungicides, to allow the design of better strategies for use of fungicides that will enhance the efficacy of control strategies. The project also investigated the biology and epidemiology of resistance to two major fungicides (benomyl and propiconazole) and less sensitive strains to improve the understanding of the effect that genes for resistance have on some important components of fitness of *M*. *fijiensis*.

Background. Results to date have shown that the genes controlling resistance to fungicides in the pathogen do not reduce its aggressiveness. It has also been confirmed that two fungicides, propiconazole and tridemorph, are synergistic in their effect on the fungus, and thus may give better control in combination. Most importantly, it is now known that the previously proposed strategies for managing fungicide resistance to the more important pesticides will not work. The practice of rotating pesticides to allow a decline in the frequency of resistance to favored pesticides will only result in failure, as the expected reduction does not occur. Apparently the expected selection forces that would cause the change to occur do not function as expected. Once resistance to a pesticide has occurred there is little hope of it once again becoming effective. This has serious consequences for banana producers worldwide.

Results

• The sensitivity of progenies from 28 crosses of isolates of *M. fijiensis* with different sen-

sitivities to propiconazole has been determined

- Acquisition of genes for resistance to chemicals does not have an effect on the fitness of the pathogen populations. Resistance patterns remain although fungicide use is stopped
- A greater understanding of the relationship between acquisition of resistance and the ability of isolates of *M. fijiensis* less sensitive to propiconazole to produce conidia in culture.

Outcome. Results from conidial sporulation between propiconazole less-sensitive, and propiconazole-sensitive strains of M. fijiensis, are important to understand the effect that gaining genes for resistance has on fitness of the pathogen. This would help to explain why some strategies, and what strategies, would help prevent or diminish the resistance population, thereby improving the control of the disease. Results from crosses and inheritance of the resistance to propiconazole will help understand the potential for triazole-resistant genes to flow in the population and, in conjunction with other aspects of fitness, will help clarify the efficacy of strategies for resistance management in the population of M. fijiensis.

Impact. Project activities have confirmed that a chemical-free period does not lessen the impact of populations of pathogens resistant to fungicides. Although this was a negative finding in some respects, it did confirm that strategies for improved use of chemicals to control the black Sigatoka pathogen are not easy to develop, and that continuing development of new chemicals, together with associated costs, is the principal avenue by which some retention of chemical control can be managed.

Banana Streak Virus (BSV)

PN14: Elimination of banana streak badnavirus (BSV) from improved Musa

germplasm and related studies on transmission and host plant/virus/vector interactions International Institute of Tropical Agriculture, Nigeria Principal investigator: Jonathan H. Crouch

Activity. This project first aimed to develop reliable therapeutic methods to eliminate BSV from improved *Musa* germplasm. This objective was later altered to the identification of *Musa* plant tissues that are free of both genomic and episomal BSV, when it was found that the virus had the ability to integrate into the *Musa* genome. The project focused heavily on the need for a greater understanding of the host plant/virus/vector relationships, and BSV transmission and identification of its vectors, when it was realized that the virus was far more insidious than originally thought. The project also undertook to provide a protocol for the elimination of BSV from infected genotypes.

Background. IITA undertook to seek more reliable methods of detection of BSV in naturally infected plants. BSV is a difficult virus inasmuch as it has the capacity to insert itself into the host genome, thus making detection difficult. IITA researchers and their collaborating partners have confirmed that the IC-PCR method is detecting viral (episomal) DNA, and they are continuing to work toward refining this detection technique. They are also continuing to gather more information about the insect vectors of the virus, and the best protocols for evaluating disease symptoms.

Results

- Diagnostic techniques were developed for BSV detection in infected banana and plantain.
- BSV transmission through suckers was proven, and transmission through true seed implicated. It was also found that materials propagated through in vitro culture were highly susceptible to the virus.

- Several mealybug species that are potential vectors were identified and colonies initiated, but transmission was not confirmed.
- The researchers developed a better understanding of the symptom expression of BSVinfected improved IITA hybrids and local landraces, under natural and controlled-environment conditions.
- The project added significantly to the knowledge base of screening for resistance and characterizing field resistance and/or tolerance.

Outcome. This project resulted in an increased understanding of BSV, its etiology, epidemiology, and possible control. As a direct result of the activities undertaken, informed decisions regarding the safe movement of germplasm can now be taken. The project has also generated knowledge that will greatly assist future breeding efforts and quarantine activities.

Impact. The project has produced increased knowledge and understanding of BSV. Information relevant to the safe movement of germplasm and appropriate quarantine activities was produced, although the virus remains a serious constraint to the movement of germplasm due to its incorporation into the genome. BSV is now also increasingly recognized as a production constraint in certain banana-growing areas.

PN18: Tolerance/resistance of bananas to nematodes

Centre Regional Bananiers et Plantains (CRBP), Cameroon Principal investigator: Roger Fogain

Activity. This project attempted to identify sources of resistance to the major nematode species of banana and plantain from the *Musa* germplasm collection at CRBP, and the provision of information on the susceptibility levels of plantain and other cooking bananas. The researchers also attempted to identify less tolerant plantains that can be recommended to farmers. The project will assist the CRBP breeding program, by providing information on the susceptibility levels of parental materials and their progenies to two of the most important nematodes of banana and plantain (*Radopholus similis* and *Pratylenchus goodeyi*).

Background. Banana and plantain are important staple foods for a significant proportion of the Cameroon population, and are an important source of export revenue. Radopholus similis is a major pest in commercial plantations at low altitudes, whereas Pratylenchus goodeyi is dominant in smallholder plantations in highland areas with cool temperatures. No control of nematodes is attempted in smallholder plantations, whereas in commercial plantations the use of nematicides is one of the main costs of production. This project established a close association with the CIRAD Musa improvement program, and made a collaborative linkage with the nematode consortium spearheaded by KUL (Project PN19).

Results

- Data collection will continue into 1999 in the trial on verification of field resistance of accessions, selected during pot experiments, for resistance to *Radopholus similis* at two sites (Njombe and Mbalmao). The sites have different soil and climatic characteristics, but *R similis* is the dominant nematode pest species found in the two locations.
- Results of the analysis of root samples collected nine months after planting confirmed the resistance of four accessions to *R. similis*, comparable to that of Yangambi Km5.

Outcome. Thirteen plantain hybrids and nine diploid clones have been compared for resistance to *R. similis*. All but one was susceptible, with one diploid showing a low level of resistance. Tests to compare resistance in tissue culture versus conventional planting material to the nematode *P. goodeyi* showed that there was

no difference between the two. The project is contributing to the pool of knowledge on the resistance of *Musa* to nematodes, the development of new procedures for the selection of *Musa* clones for resistance to lesion nematodes, and in the identification of new sources of resistance to *R. similis.* Sources of identified resistance are available to breeding programs.

Impact. As well as contributing to our overall knowledge on resistance, results of this project will impact mainly on the breeding program at CRBP. This will enable the selection of hybrid parents displaying resistance to nematodes, thereby increasing the rate of genetic gain in level of resistance. A number of the selected clones also display resistance to other banana pests and pathogens, enhancing the possibility that multiple resistance can be achieved. The development of an early screening method will help in the rapid selection of resistant material.

PN19: Identification of durable nematode resistance sources in banana and plantain Katholieke Universiteit Leuven (KUL), Belgium Principal investigator: Dirk de Waele

Activity. This project was aimed at the identification of durable nematode resistance sources in banana and plantain. This would lead to improved nematode management beneficial to the producer and the environment, increased yields, decreased production costs, and a reduced number of applications of nematicides. The researchers hoped to provide a nematological component to existing breeding programs, and to screen banana and plantain genotypes for nematode resistance based on the development of early, rapid, and reliable screening methods.

Background. Nematodes constitute an important limiting factor of banana and plantain throughout the world. The high cost and toxicity of nematicides underlie the urgent need to

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identify nematode-resistant sources, and an early and rapid screening method for use in breeding programs. The principal investigator of this project was active in obtaining cofinancing through the Belgium Administration for Development Cooperation (BADC) and the Flemish Association for Development Cooperation and Technical Assistance (VVOB). This helped fund nematological research associates at breeding programs in FHIA Honduras, IITA in Nigeria and Uganda, VASI in Vietnam, and recently CORBANA, Costa Rica. Project staff also worked closely with PN21 in Spain during the initial year of activities. More recently, a memorandum of understanding was signed to establish closer collaboration with CRBP in Cameroon.

Results

- Six *Radopholus similis*, 3 *Pratylenchus coffeae*, and 6 *Meloidogyne* spp. populations were established for screening experiments.
- Thirty greenhouse experiments were conducted at KUL, and 75 Musa genotypes were evaluated for resistance to these populations.
- Host plant responses obtained by early greenhouse screening (in soil in pots) have been compared with host plant responses under field conditions.
- Host plant responses of in vitro micropropagated plants screened in the greenhouse were compared with the host response of suckers. Preliminary experiments demonstrated that resistance can be observed under in vitro conditions, but due to contamination of the nematode population, this screening method has been compromised.
- Multiple nematode species resistance screening of *Musa* genotypes from various sources was undertaken with no new sources of resistance found.
- Several genotypes were identified that are much less susceptible to *R. similis* compared with a susceptible reference genotype.

Outcome. This work will continue following the completion of BIP, with information being generated on the host response of *Musa* genotypes to infection at different levels of nematode screening. New techniques are currently being investigated to obtain contamination-free *R. similis* for the routine in vitro screening of *Musa* germplasm for nematode resistance. Work also continues at KUL on the host response of *R. similis* of a series of diploids and triploids from the Philippines. Although work continues, the data collected to date already enable a more reliable interpretation of the host response of *Musa* genotypes to nematode infection.

Impact. A major impact was the establishment of the Musa Nematologists' Consortium, which will continue to function following the completion of BIP. It is envisaged that additional partners from South America, the Caribbean, Africa, Asia, and Australia will be members in the near future. For banana production, the consortium provides a reliable nematological evaluation under different agroecological conditions of existing or new varieties. Under the project, KUL was able to assist FHIA in the evaluation of the genotypes used or produced in their Musa breeding program. This is the first time that nematode resistance of these genotypes has been reliably and thoroughly documented, allowing the identification of the most interesting parent lines for crossings aimed at nematode resistance. Valuable information has also been obtained on the host response of Musa genotypes to nematode infection at the different levels of nematode screening: in vitro, under greenhouse conditions, and in the field.

PN20: Identification of durable pest and disease resistance sources in banana and plantain Fundacion Hondureña de Investigacion Agricola (FHIA), Honduras Principal investigator: Philip Rowe

Activity. In collaboration with KUL, Leuven, the researchers sought to improve nematode management on banana and plantain to benefit both the producer and the environment through yield increases, production cost decreases, and a reduction in the use of nematicides.

Background. Nineteen genotypes of *Musa* have been screened for resistance to *R. similis* and *P. coffeae*, the two major nematode pests. Some genotypes with a high degree of resistance to one or both were identified for further resistance breeding. Results showed that tissue culture plants could be used to differentiate between different degrees of resistance and tolerance in the genotypes tested.

Results

- Carrot disk cultures of *R. similis* and *P. coffeae* are being maintained in the laboratory for a continuous supply of nematodes for inocula to infest pests in the shadehouse trials.
- Six shadehouse trials were completed, and four further trials are underway that include 29 genotypes.
- Data collection and management of two field trials established in 1997 continued with 17 test genotypes.
- Data from the completed shadehouse and ongoing field trials were entered into a computerized database and statistical analysis is in progress.
- The multiplication of 22 additional genotypes from FHIA's collection was started in the tissue culture laboratory for use in the final phase of the project.

Outcome. Technicians were trained to evaluate nematode resistance in subsequent hybrids developed in the ongoing breeding program. Most of the genotypes available at FHIA have been evaluated for resistance and tolerance to *R. similis* and *P. coffeae*, and are undergoing reevaluation because results varied with type of planting material, and the test was not performed in optimal conditions. Field trials should provide the necessary information to determine if early testing in shadehouse conditions is a reliable tool for nematode resistance screening. This knowledge will assist in the selection of improved genotypes for breeding programs, resulting in varieties with increased yield, lower production costs due to reduced use of nematicides, and environmental benefits.

Impact. Prior to BIP there had been no validation of resistant material in breeding programs. The information from this project provided knowledge of resistance when selecting parents for crossing. New sources of resistance to nematodes were found, having the potential to increase knowledge on the mechanism for resistance. This could have considerable value if a biotechnological approach is considered for resistance. Evaluations will continue, linked within the nematology consortium.

PN21: Identification of durable nematode resistance sources in banana and plantain Institut de Recerca i Tecnoligia Agroalimentaries (IRTA), Spain Principal investigator: Jorge Pinochet

Activity. The specific aim of this project was to establish a live collection of migratory endoparasitic and root-knot nematodes of the main nematode species that attack banana and plantain. A second activity was the preparation of banana plant material for resistance evaluation, and the initiation of screening trials under greenhouse conditions.

Background. The project was initiated as a single year component of the nematode consortium, with the same objectives.

Results. Twenty-seven isolates comprising three genera and six important *Musa*-attacking nematode species (*Radopholus similis, Pratylenchus goodeyi, P. coffeae,* and *Meloidogyne* spp.) were isolated and established in monoxenic cultures, with the exception of *Meloidogyne* spp.,

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which were established and maintained on alternate hosts.

Plant material and screening tests of *Musa* germplasm of interest to the Canary Islands against *Pratylenchus goodeyi and Meloidogyne* spp. under greenhouse conditions were organized.

Outcome. A pure, abundant, and highly infective inoculum was made available for plant material evaluation and disseminated to consortium researchers for use in screening trials.

Impact. As part of the nematode consortium activities, the germplasm screening for resistance will enable more effective breeding programs to be initiated through selection of resistant material. This development should reduce costs of nematode control and lower the use of nematicides.

4

BIP Impact Assessment

P. D. Chudleigh

This report provides a brief assessment of the impact of the Banana Improvement Project (BIP) cosponsored by the Common Fund for Commodities, the FAO Inter-Governmental Group on Bananas, and the World Bank. BIP consisted of 18 individual projects funded between 1994 and 1998. The intended beneficiaries were producers of dessert bananas, particularly smallholders, with potential spillovers to plantain producers. The principal objective was to increase banana production through the development and use of higher-yielding, diseaseresistant varieties, and by reducing production costs, especially for pesticide applications.

BIP was managed by the World Bank, with a part-time Program Manager and a full-time Project Coordinator, and a Scientific Advisory Panel. The Panel was involved in industry consultation, the definition of research priorities, and project selection. The Panel also met regularly to review the progress of the portfolio after projects had commenced.

Before the projects were called for and selected, the World Bank commissioned a report to investigate the then current status of banana research and priority areas for BIP. Buddenhagen (1996) highlighted the need for combining innovative breeding approaches with advances in plant biotechnology. This influenced the composition of the project portfolio.

Most of the individual research projects fell into two categories, breeding and biotechnology, and many were long term and strategic in nature, where knowledge was the primary output. The knowledge produced has been in the following areas:

- The collection and characterization of a wide range of banana germplasm that will allow increased effectiveness of the banana breeding programs around the world
- Increased efficiency of breeding programs through improved testing, screening and typing methods and development of protocols; use of additional information assembled through use of genetic markers, particularly for disease resistance to Sigatoka, Panama disease, and nematodes
- Improved and refined technology for genetic manipulation including transforma-

tion processes for banana and improved knowledge of disease resistance mechanisms in banana.

The use of this knowledge will provide positive outcomes for plantain and all types of banana production, whether for cooking, domestic consumption as dessert banana, or for production of export banana. However, the application of this knowledge in the future to provide tangible outcomes for producers and exporters will require further R&D investment.

Although the program did not specifically target applied outputs, there was a number of areas where the program has had or will have direct industry impacts in the short term. These include some black Sigatoka resistant clones produced in BIP currently being used to assist control of the recent black Sigatoka incursion into the Amazonas region of Brazil. Also, the ability to now type strains of *Fusarium* in a given location when considering new banana developments will be beneficial to new banana enterprise developments.

In summary, BIP has provided a unique set of outputs in terms of both content and process. BIP has produced knowledge of a strategic nature as well as some technologies that have been, and can be, applied in the short term. In terms of process, the competitive bid system for identifying the best projects to fund within a strategic vision has proved successful, as has the leverage of resources into the banana R&D world, as well as increased collaboration and coordination among research teams.

Outcomes of BIP are defined as the activities or events that have occurred, or are likely to occur, as a result of the outputs from BIP. Outcomes are directly relevant to producers of banana and to others in the banana value chain including consumers. Outcomes can be positive or negative and are usually associated with benefits and costs. These benefits and costs may fall differentially on the various participants in the banana value chain.

Two important short-term outcomes stand out. The first was the ability to type *Fusarium*

strains before locating new banana developments. The worldwide collection of the various strains of Fusarium will serve as a reference collection in the future enabling the strains causing an outbreak of Panama disease to be quickly identified. New banana developments can be more safely implemented through determining the strains present in any given location before planting begins. For example, recent outbreaks of Fusarium wilt in Cavendish plantations in the eastern tropics (peninsular Malaysia, Sumatra and Halmahera) caused enormous damage. These outbreaks have ominous implications for the durability of western trades that are based on Cavendish clones. The avoidance of future losses will be one of the short-term benefits from BIP.

The second was the Brazilian genetic material used in Amazonas to combat the spread of black Sigatoka as it enters Brazil. Some banana clones resistant to black Sigatoka have been produced in Brazil with assistance from BIP and are being used to slow the rate of advance of the disease in northern Brazil. This would have a major reduction in the costs of those who can potentially afford to spray with fungicides as well as reduce yield losses of those who can not afford chemical control technologies.

While the short-term outcomes and associated benefits are likely to have been significant, the most important outcomes and contributions from BIP are likely to be derived from the strategic knowledge contributions of BIP.

Outcomes associated with knowledge generated from BIP are inherently uncertain. However, in terms of assessing the impact of BIP, it is possible to make some assumptions about the future, and the role that BIP will have played in the future development of the export banana industry.

The key events shaping the future use of knowledge from BIP with respect to export banana industries would include the development of (in full or in part) a Cavendish or alternative export banana type that is resistant to disease. Benefits that might be attributed at least in part to the BIP can be grouped into economic, environmental and social, and health benefits.

The principal economic benefit most likely associated with the development of a diseaseresistant banana is the reduced cost of production of export bananas through chemical replacement. The principal environmental benefit associated with BIP is the likelihood of lesser amounts of pesticides being used if resistant or more tolerant varieties of export bananas are developed. The lower the pesticide applications, the lower the potential damage to the environment. As with the environment, a range of statements has been made that fungicides and nematicides used in banana production are associated with negative health impacts on banana plantation workers, and those living in banana-producing communities. Some of the perceived benefits related to health that would arise from developing disease-resistant banana would be improved health of banana industry workers and higher labour productivity, as well as improved health and quality of life of communities in banana-producing regions.

To evaluate the past investment in BIP in quantitative terms, it is necessary to construct future scenarios that assume that the particular knowledge generated from BIP is used in further R&D efforts that are eventually successful, with the resulting technologies or products being adopted by industry. This requires a number of assumptions to be made about the type of future investment and associated costs, the time period before disease- resistant bananas will actually be produced, the likelihood that the technologies will be produced within the time frame assumed, and the proportion of total benefits that might be attributed to BIP.

The two key future scenarios assumed were:

1. The production of a transgenic Cavendish that is reasonably resistant to black Sigatoka and nematodes

2. The development of export banana types that are resistant to black Sigatoka and nematodes. This is assumed to be achieved through a combination of transformation and regeneration technologies together with breeding components and has resulted in a robust form of resistance.

Each of these scenarios was analyzed in terms of potential benefits and costs that might be expected "with" and "without" the R&D program.

The analysis was effected through a discounted cash flow analysis that ran for a period 35 years, commencing in the 1994 calendar year. All costs and benefits were discounted back to 1994 using a discount rate of 7 percent. All values were expressed in 1998 U.S. dollars. Investment criteria estimated included the Net Present Value (NPV), the Benefit-Cost Ratio (B/C Ratio), and the Internal Rate of Return (IRR). Criteria were estimated for each of the two scenarios separately.

The investment criteria estimated using the base assumptions for scenarios 1 and 2 are summarized below:

Expected Investment Criteria for Scenario 1 (Transgenic Cavendish) (discount rate 7%)

	NPV	B/C	IRR
	(\$ million)	Ratio	(%)
Total investment	578	9:1	21.4
BIP investment only	27.1	10:1	21.9

Expected Investment Criteria for Scenario 2 (Alternative Banana Type) (discount rate 7%)

	NPV	B/C	IRR
	(\$ million)	Ratio	(%)
Total investment	484	8:1	20.0
BIP investment only	22.8	8:1	20.5

The estimation of benefits is conservative in two ways. First, the actual parameters used in the quantitative analysis could be considered conservative. Second, there are a number of other benefits outlined in earlier chapters that have not been included in the quantitative analysis. Environmental and human health benefits are two such benefits that are usually difficult to value but are likely to be high and

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significant. Such benefits could also provide justification for public investment in banana improvement, since benefits could be captured by communities as opposed to the private sector in banana-exporting countries.

Given the assumptions made in the analysis, the results suggest that BIP could be expected to provide a rate of return on funds invested of at least 20 percent. Conservative assumptions have been used through the analyses, and the sensitivity analyses and break-even parameters estimated show that the results are robust across a credible range of assumptions. The results of the investment analysis demonstrate that the payoffs being sought in terms of disease-resistant bananas can justify a large investment in banana improvement research.

BIP has provided the methods for transformation and regeneration of bananas, and has moved the world banana industry closer to the reality of a transgenic banana. Transformation and regeneration have now been accomplished. There has also been significant progress demonstrated in terms of new germplasm for use in breeding programs and methods for making breeding programs more effective in the future. The most important output from BIP was strategic knowledge that can be used in further R&D projects in the future.

Background and Methods Employed

I was asked by the World Bank to do an impact assessment on the results of the contracted research projects of the Banana Improvement Project, on the future production techniques and costs of the export banana industry. The terms of reference required that I analyze and summarize Project and individual project documentation; develop a framework for benefit-cost analysis of the Project; visit selected locations to meet with principal investigators; and interact with members of the Scientific Advisory Panel, and visit the World Bank to give an initial report of findings.

Most of the information assembled in this report was gained from progress and termina-

tion reports for individual projects, made available by the Program Coordinator before and during my visit to the World Bank. In addition, I visited project sites (two each in Australia, Belgium, and France), for discussions with principal investigators. I also met the three members of the Scientific Advisory Panel, and further exchanges with Advisory Panel members also took place. The consultations and preparation of this report were done in October 1998.

In addition, people associated with the world export banana industry were also contacted to assist with various information requirements for the assessment.

Aims, Objectives, and Priorities

The goal addressed by BIP is to make banana production more profitable and to reduce the use of pesticides. The aim of the Project is to increase the productivity of the crop through the development and use of varieties that are higher yielding and disease resistant, and by reducing production costs, especially for pesticide applications.

The two key objectives of BIP are to support research that will:

1. Develop and evaluate a range of improved banana varieties with export potential, incorporating increased productivity and durable disease resistance through conventional and nonconventional breeding techniques

2. Develop more efficient and integrated disease management practices, especially for black Sigatoka disease.

The objectives of BIP are consistent with the strategies of the FAO/IGB which included:

- Making bananas a more profitable crop for smallholder producers, by increasing productivity and reducing production costs especially through improved disease control based on durable disease resistance
- Reducing pesticide use, thus reducing costs of production and limiting environmental

damage and human health risks caused by excessive use of fungicides and nematicides

- Improving the reliability of supply of highquality product
- Expanding the export market, including opening up niche markets for specialty bananas
- Improving access to markets.

Projects Funded

Before the projects were called for and selected, the World Bank commissioned a report in 1994 to investigate the then current status of banana research and priority areas for BIP. Buddenhagen (1996) highlighted the need for combining innovative breeding approaches with advances in plant biotechnology. This influenced the composition of the BIP portfolio of projects.

The projects funded concentrated on banana improvement rather than agronomy or integrated pest management. Both breeding and biotechnology projects were funded.

A list of projects funded within BIP is provided in Chapter 3.

Type of Research

The list in Chapter 3 also includes my rating of strategic or applied, and whether the project was biotechnology or breeding oriented. A project was defined as strategic when the intended output from the project could not be used directly by industry at the present time, and where the output was intended as an input to further research. On the other hand, a project was considered applied if the outputs could be used directly by industry. Some projects had a mix of both applied and strategic components.

Strategic projects usually produce knowledge as their primary outputs. Such information or knowledge can be used in other projects to eventually produce applied outputs that are implementable by industry. Strategic projects are usually long term in nature. As can be seen from the listing in Chapter 3, the portfolio of projects funded by the program is predominantly strategic.

Another observation was that projects focused more strongly on the first objective of the program (developing the improved banana types) compared with the second objective (more efficient and integrated disease management practices for black Sigatoka).

Principal Outputs of BIP

The outputs of BIP have been:

- Increased knowledge that will be used in further projects targeted toward producing disease-resistant export bananas
- Some applied and more immediate outputs that can immediately be used by industry
- Leverage of additional resources into banana R&D, and increased collaboration and international coordination in banana plant improvement R&D.

Strategic Knowledge

Strategic knowledge has been the principal output from BIP in the following areas:

- Collection and characterization of a wide range of banana germplasm that will allow increased effectiveness of the banana breeding programs around the world (PN3 and PN4)
- Increased efficiency of breeding programs through improved testing, screening, and typing methods and development of protocols; use of additional information assembled through use of genetic markers, particularly for disease resistance to Sigatoka, Panama disease, and nematodes (PN1, PN2, PN14, PN15, PN18, PN19, PN20, and PN21)
- Improved and refined technology for genetic manipulation including transformation processes for banana, and improved knowledge of disease resistance mecha-

nisms in banana (PN5, PN8, PN9, PN10, PN11, PN12, PN14, and PN18).

The use of this knowledge will provide positive outcomes for plantains and all types of banana production whether for cooking, domestic consumption as dessert bananas, or for production of export bananas. The application of this knowledge in the future to provide tangible outcomes for producers and exporters is addressed later.

Applied Outputs

Although BIP did not specifically target applied outputs, there were a number of areas where the Project may have direct industry impacts, albeit not necessarily in the export banana industries. These include projects PN3, PN5, PN15, PN17, and PN22.

PN5 and PN22 were projects embedded in the breeding programs of EMBRAPA (Brazil) and FHIA (Honduras), respectively. Black Sigatoka clones produced in the BIP-supported part of the EMBRAPA breeding program are currently being used to help control the recent black Sigatoka incursion into the Amazonas region of Brazil. Also, the FHIA attempt to develop disease-resistant bananas with a short stature could result in such types being commercially accepted.

Project PN3 evaluated Indian germplasm, and the results may have implications for the use of more short-statured bananas and a cost reduction in propping in south Indian production systems.

Project PN15 will have direct application by industry in being able to type strains of *Fusarium* in a given location when considering new banana developments.

The finding in Project PN17 concerning the continuation of resistance to chemicals once fungicides are no longer applied is of direct industry relevance.

These applied outputs should be viewed as direct benefits from the BIP investment, and are

considered again later in a description of outcomes.

Leverage

Other investments in banana research have been leveraged by BIP. This has been achieved within and outside many of the 18 projects. For example, the establishment of BIP has encouraged the Flemish Association for Development Cooperation and Technical Assistance (VVOB) and the Belgium Administration for Development Cooperation (BADC) to provide significant funding to the nematode consortium, through positioning scientific personnel in various breeding programs around the world. This will continue after BIP is completed.

A conservative estimate of the funds contributed to BIP projects by the host institutions as cofinancing is US\$2 million.

Other instances of leverage and attraction of other resources into banana research include New Zealand support in Vietnam for postharvest production studies, and additional funds outside the original project contributed by the University of Hong Kong and the University of Hawaii.

Coordination and Collaboration

Because of the nature of the Project, there was enhanced coordination of effort and increased collaboration in international banana plant improvement. This was particularly evident in relation to what was termed the "biotechnology consortium" (a joint effort between KUL, BTI, and QUT). This alliance fostered staff exchanges between the three institutions involved, and assisted the transfer and refinement of particle bombardment and Agrobacterium transformation and regeneration technology. However, in the latter stages of BIP the level of activity diminished. This was due to the pursuit of more specific goals by each institution involved in the biotechnology initiative within BIP.

Increased collaboration was also fostered in nematode screening for resistance through what was termed the "nematode consortium." This consortium will continue after BIP is completed, and will be important in the screening and understanding of nematode resistance in the future (de Waele 1995; Speijer and de Waele 1997).

A number of other collaborative efforts have been stimulated by BIP. These include continuing interaction between Australian and Vietnamese scientists, and the Australian involvement with testing genetic material for a number of breeding programs. Unfortunately, attempts to integrate the classical breeding approaches in Honduras with molecular aids to breeding have not succeeded. Integration between the FHIA approach to breeding and that of the French breeding program would have been beneficial to both areas of endeavor.

BIP was successful, through the competitive grants approach, in attracting new research partners into banana improvement. Moreover, due to the necessity for many research institutions to attract external funding, such as through BIP, the attraction of more resources into banana plant improvement was achieved. Although some of the resources attracted into BIP projects would have probably been committed to banana improvement anyway, it is likely that, due to BIP, significant additional resources were allocated. Two examples:

1. CIRAD has now secured an additional permanent staff position for banana improvement due to the program.

2. Generic resources provided by VVOB in Belgium have been committed to banana improvement through the nematode consortium.

Conclusion

BIP has provided a unique set of outputs in content and process. The Project has produced strategic knowledge as well as some technologies that have been, and can be, applied in the short term. The competitive bid process to identify the best projects to fund within a strategic vision has been successful. The leveraging of resources into banana R&D and increased collaboration and coordination among research teams, have also been important achievements.

Actual and Potential Outcomes and Benefits

Funding often dictates rigid dates for startup and completion of projects, but the research process cannot be entirely controlled by the calendar. Some of the BIP-funded projects must therefore continue into 1999, and others will be continued by the research institutions. It is important that results be reported, even after the official end of BIP in 1999. How this will be achieved is unclear, but it does highlight the unsatisfactory nature of short-term funding such as that provided by BIP.

Outcomes of BIP are defined as the activities or events that have occurred, or are likely to occur, as a result of the outputs from BIP. Outcomes are directly relevant to producers of bananas, and to others in the banana value chain, including consumers. Outcomes can be positive or negative and are usually associated with benefits and costs. These benefits and costs may fall differentially on the various participants in the banana value chain.

Outcomes can be short term or long term. Short-term outcomes are often preferable but not always easy to achieve in agricultural research.

Outcomes can be classified into those of content (products, technologies, knowledge), and those of a structural nature (for example changed priorities and resource allocation, attraction of additional resources).

The following describes the outcomes and associated benefits and costs that have arisen due wholly or partially to BIP.

Short-Term Outcomes and Benefits

Two important short-term outcomes of BIPsponsored research are:

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1. The ability to type *Fusarium* strains before locating new banana developments

2. The Brazilian genetic material used in Amazonas to combat the spread of black Sigatoka as it enters Brazil.

Typing Fusarium strains. The worldwide collection of the various strains of Fusarium will serve as a reference collection in the future, enabling the strains causing an outbreak of Panama disease to be quickly identified. New banana-growing areas can be more safely implemented through determining the strains present in any given location before planting begins. For example, recent outbreaks of Fusarium wilt in Cavendish plantations in the eastern tropics (peninsular Malaysia, Sumatra, and Halmahera) caused enormous damage. These outbreaks have ominous implications for the durability of international trade that is based on Cavendish clones. The avoidance of future losses will be one of the short-term benefits from BIP.

Brazilian material. Some banana clones resistant to black Sigatoka have been produced in Brazil with assistance from BIP and are being used to slow the rate of advance of the disease in northern Brazil. This will result in significant savings to producers who use sprays. This will also reduce yield losses for resource-poor farmers who can not afford to buy fungicides.

Long-Term Outcomes and Benefits

Although the short-term outcomes and associated benefits are likely to be significant, the most important outcomes and contributions from BIP are likely to be derived from the strategic new knowledge.

Outcomes associated with knowledge generated from BIP projects are inherently uncertain. In assessing the impact of BIP, however, it is necessary to make some assumptions about the future, and the role that BIP may have played in the future development of the export banana industry. The key events shaping the future use of knowledge from BIP by export banana industries would include: development (in full or in part) of a Cavendish or alternative export banana type that is resistant to black Sigatoka and Panama disease, as well as to nematodes and viral diseases. These developments may use conventional breeding biotechnologies such as genetic markers or genetic engineering.

The key uncertainties associated with developments of this type would include:

- The number of years until they occur
- The annual investment required for them to happen within a given period with a specific probability of research success
- The applicability of the resistant material (across pathogens and strains within a pathogen)
- The durability of resistance inherent in the new material
- The level of use of chemicals still required
- Any interaction or enhancement with cultural techniques including IPM
- The level of maintenance of resistance through breeding/biotechnology required on an ongoing basis after the events are realized
- The cost and level of availability to industry of any new material produced
- The level of adoption of new export banana types.

There are still serious constraints to effectively introduce disease-resistant genes and have them expressed in banana plants. A major constraint continues to be the degree of somaclonal variation in the recipient banana material. The cell target tissue needs to be quickly and effectively reproducible in vitro to reduce the degree of variation.

Promoters must be more specific so that they are not expressed in all tissue types, including the fruit. Specific promoters that are only expressed in certain tissues (for example roots), or when an infection is present, are required; however, it is not known how precisely specific promoters need to be.

Target genes need to be identified and evaluated so that they can be combined in a manner that confers robust protection.

Finally, it is not yet known how strongly expression of the gene in the laboratory will be related to resistance in the field.

These technical issues will influence the time, resources, and probability of success of the events actually occurring. For example, time and resources will vary depending on whether a short-term solution is assumed, or one of a more durable nature incorporating multiple sources of resistance.

There are also considerable commercial constraints to the development of transgenic or disease-resistant bananas. One of these is the intellectual property issue (Lele, Lesser, and Horstkotte-Wesseler 1999). Viral transfer constraints (banana streak virus) (Frison and Sharrock 1998), and the need to test against multiple strains of black Sigatoka, will also reduce the rate of adoption of any transgenic banana across the whole export industry.

In addition, there are legal issues that might retard progress in introducing a transgenic banana into the field. These include consumer concern about genetically engineered food crops, a concern that is likely to diminish with education and clear labelling. The reduction in chemical use associated with the production of genetically engineered banana will help offset some of the negative perceptions.

If the transgenic banana is not a Cavendish type, there may be additional difficulties of market development and a slower rate of adoption throughout the industry.

The greater the investment in biotechnology R&D, the broader and more robust may be the resulting form of resistance. Pathogens can become resistant to gene products, especially if the transgenic banana plants are used intensively and the genes bestowing the resistance properties are not broad based in their action. Combining different forms of resistance in one or more transgenic plants, along with the use of some chemical spraying, is likely to provide a more enduring strategy. In any case, the extent of spraying is likely to be considerably reduced.

Resistance to *Fusarium*, however, is likely to be more robust because the fungal organism in this case has less chance of recombination due to its asexual form of propagation.

Although Sigatoka-resistant genetic material should result in significant savings because of reduced use of chemicals, and significant quality improvements in production for domestic markets, the technology is not likely to be made available outside the private interests that are most likely to develop the clones in the first instance. If the development is through the public sector, there will still be royalties owing to the owners of the embedded technologies. This may result in significant costs for the resistant planting material. Any increase in the cost of planting material is still likely to be less than the savings in chemical costs, thereby conferring a net benefit to the industry. Society will benefit in two ways: (a) improved environment with the reduction in chemical use, and (b) improved health of industry workers who will be handling less toxic chemical material.

The following provides a qualitative description of the type of long-term benefits that might be attributed in part to BIP. The benefits are classified as economic, environmental, and social and health.

Economic Benefits. The principal benefit most likely to be associated with such key events as specified earlier is the reduced cost of production of export bananas. This might be achieved through:

- Reduced chemical spraying costs
- Reduced importation costs of chemicals in banana-exporting countries
- Higher yields (which can be translated into a cost reduction)
- Higher quality fruit resulting in less wast-

age along the value chain (which can be translated into a cost reduction)

- Reduced other control costs such as saved roguing costs (BBTV), or lowered replanting and relocation costs (Panama disease)
- The ability to reclaim land lost to banana production by disease.

The economic benefits should accrue to large plantations producing for export, and to smallholders producing for local markets. Smallholders often cannot afford chemical treatments, so losses to disease such as black Sigatoka can be significant. With higher productivity, less land may be required to produce a given level of banana production or more bananas can be produced, which will result in improved profitability to individual producers, regions, and countries.

The replacement of chemicals is required due to the ever-increasing costs of chemical control. Some chemicals have been, or are being, banned from use. Resistance to chemicals is increasing and new ones have to be developed at considerable cost.

Other benefits can flow on from a more profitable banana export industry, since it makes an important contribution to export income in a number of countries. Improved consumer confidence, and a higher level of food security in banana-consuming countries because of the reduction in chemical use, will be beneficial to banana producing and exporting countries.

Environmental Benefits. The principal environmental benefits associated with BIP-supported work are those resulting from the lower amounts of pesticides applied, if resistant or more tolerant varieties of export bananas are used or if a more efficient chemical regime is identified. The lower the pesticide applications, the lower the potential damage to the environment.

Environmental damage has not been precisely quantified for the whole of the banana export industry around the world, but there is

documentation on specific countries suggesting that at least some negative impact is occurring (Andreatta 1997; Colburn 1997). In addition to ecosystem damage, and possible reduction in biodiversity, there is at least one instance of a potential negative impact on another export industry through fungicide runoff into prawn farming locations in Ecuador (Colburn 1997). Short-term impacts on the environment are serious, but can be dealt with more easily than potential long-term impacts, which are uncertain and whose impact is less focused and more widespread. In both cases, there is difficulty in dealing with these issues because government policies in many banana-exporting countries tend to create a conflict between economic development goals and environmental sustainability.

The objectives of BIP, developing diseaseresistant types with an attendant reduction in chemicals, take on even more importance if the political and government policy vehicles are incapable of resolving such issues.

Hence benefits from even partial success in developing resistant banana varieties should have a positive environmental impact through:

- Reduced impact of chemicals on industries relying on water quality in rivers and estuaries (for example fishing, prawn farming)
- Reduced impact of chemicals on other agricultural industries
- Improved conservation of ecological systems in banana-producing countries with associated amenity and esthetic benefits
- Improved environment for tourism activities
- Reduced need for increased land areas for bananas thereby potentially conserving native forests.

Social and Health Benefits. Fungicides and nematicides used in banana production are implicated in health problems of banana plantation workers, and those living in bananaproducing communities. This is due to the use of various chemicals, and particularly those applied through spraying, where overuse and misuse is reported. Swennen (1997) claims that people associated with banana production have a 10 percent higher chance of damage to their vital organs. Andreatta (1997) and Murray (1994) suggest that chemical use in banana production is associated with human health problems.

Developing disease-resistant bananas and the reduction in use of chemicals would result in improved health of banana industry workers and higher labor productivity. There would also be an improvement in health in communities generally, with an attendant increase in the quality of life in banana-producing areas.

Other social improvements from increasing productivity in the banana industry would include the maintenance of, or increase in, employment.

Process and Structural Outcomes and Benefits

A number of process benefits have arisen from the BIP investment. As mentioned earlier, there has been leverage of additional resources into banana research, as well as improved coordination and collaboration between research scientists. These outputs should contribute to more effective investment in banana improvement in future, and should result in lessening the time that it might take to produce disease-resistant types in the future.

A Framework for Analysis

The following framework has been provided as a guide to analyzing the value for money obtained by CFC in its investment in BIP. Effecting an investment analysis for a program of what is really strategic research is not a simple or easy undertaking. However, it is possible to effect a quantitative analysis using some of the benefits that were described above.

Clearly there is a wide range of scenarios for the future. Assessing the impact of the past

BIP investment will require assumptions about the future, and needs to take into account the uncertainty concerning the success and timing of any scenarios postulated.

Potential gross benefits are those that can be derived from the outcomes, such as the development of disease-resistant export banana. Additional costs involved in realization of defined potential gross benefits need to be subtracted from the potential gross benefits. For example, additional costs of planting new resistant material that might arise due to intellectual property rights for transgenic material, or due to the additional value chain costs of exporters changing to alternative export banana types, would need to be subtracted from the production cost reductions. Once additional costs incurred are subtracted, the remaining benefits can be termed potential net benefits.

Potential net benefits need to be assessed relative to what would have been likely to happen to industry costs in the event of these positive outcomes not having taken place at some stage in the future. A "no intervention" strategy where there is no R&D investment is reasonable to assume. In this case, assumptions would have to be made about escalating costs of chemical control, banning of nematicides, or increasing environmental impacts over time.

The term "potential net benefit" is used to signify that the benefits may not necessarily be realized. When multiplied by some estimate of the probability of the event actually occurring, then the resulting parameter may be termed "expected net benefits." Expected net benefits are those that might be realized by the industry and consumers of export bananas.

The distribution of benefits to producers, exporters, retailers, and consumers is not part of the framework specified here. Such distributional aspects require a more sophisticated framework and more detailed assumptions than can be made in the current impact assessment.

Expected net benefits may not be fully realized if the technologies produced are not applicable to all geographic regions or all export banana industries, all pathogen strains, and other factors. The actual benefits that might be ascribed in part to BIP will depend on how the technologies are actually adopted by industry. In other words, even though expected net benefits to multinational companies might be positive, there may be other constraining factors that might retard or negate the technologies being adopted by industry. Hence the rate of adoption of any ensuing technologies needs to be taken into account.

Investment Analysis

Approach

To evaluate the past investment in BIP in quantitative terms, it is necessary to construct future scenarios that assume that the particular knowledge generated from BIP is used in further R&D efforts that are eventually successful, with the resulting technologies or products being adopted by industry. This requires a number of assumptions to be made about the type of future investment and associated costs, the time period before disease-resistant bananas will actually be produced, and the likelihood that the technologies will be produced within the time frame assumed.

A part of the benefits that might arise in the future can then be attributed to BIP and the remaining benefits to non-BIP investment. Assessing the relative contributions is difficult. One way is to ascertain the proportion of the BIP investment to the total investment that will be required, and attribute benefits to BIP according to that proportion. This attribution method was the principal method used in the investment analysis described.

Another way in which the likely returns to the BIP investment can be estimated is through an assumption that BIP "sped up" the delivery of the final products that are achieved. For example, it could be assumed that the development and commercialization of a disease-resistant export banana would have occurred in the year 2017, and that the additional investment and impetus provided by BIP would allow it to occur in 2012. An exercise using this method has also been used in the present analysis, and is hereafter known as the "earlier impact" method. In this case it is assumed that investment in R&D would have occurred without BIP, but that BIP contributed to the effectiveness of the mainstream R&D investment, through a speeding-up of the realization of scenarios envisaged, (that is the benefits from the transgenic and alternative banana introduction would have been received earlier).

The two key future scenarios specified earlier were:

1. The production of a transgenic Cavendish that is reasonably resistant to black Sigatoka and nematodes

2. The development of export banana types that are resistant to black Sigatoka and nematodes. This is assumed to be achieved through a combination of transformation and regeneration technologies, together with breeding components, and has resulted in a robust form of resistance.

For the application of the attribution method, each of these scenarios is analyzed in terms of potential benefits and costs that might be expected "with" and "without" the R&D program. In the case of benefits for the "with" R&D situation, these are principally the reduction (not necessarily elimination) in chemical costs and any reduction in yield losses from improved disease control. These benefits may differ in each of the two scenarios analyzed.

In the case of costs for the "with" R&D situation, these will include the costs of R&D to develop the disease- resistant banana. In the case of the necessity to move away from Cavendish (scenario 2), it will also be necessary to include the cost to producing and exporting systems of changing to another banana type.

These benefits and costs for the "with" R&D situation are then compared with the "without"

R&D situation where, while there are no R&D costs, there are assumed to be future increases in chemical costs and yield losses.

In the case of the "earlier impact" method, the costs of BIP were placed against the difference in benefits expected in two situations. The benefits in the "with BIP" situation were assumed to commence in 2012, but in the "without BIP" scenario, the benefits were assumed to commence later. It is the difference in the benefits between these two situations that is assumed to be the benefit due to the BIP investment.

Assumptions

Assumptions used in the analysis using the attribution method are summarized in Table 4.1.

The cost of applying for and holding patents was assumed to be only US\$1-2 million and was not included in the analysis due to its relative insignificance in comparison with other costs. However, the cost of royalty payments was considered potentially significant. Such royalty payments could be considered part of the total benefits estimated in the analysis. Assuming such royalty payments do not inhibit the adoption of the technologies, the total benefits will remain the same but may not accrue solely to banana producers, exporters, or banana consumers. No estimate has been made of the size of any royalty payments or the distribution benefits between the banana export industry, and research organizations or biotechnology companies. There has also been no attempt to distribute the total benefits that might accrue between banana producers and consumers.

The results were obtained through a discounted cash flow analysis that ran for 35 years, commencing in the 1994 calendar year. All costs and benefits were discounted back to 1994 using a discount rate of 7 percent. All values were expressed in 1998 U.S. dollars.

Investment criteria estimated included the net present value (NPV), the benefit-cost ratio (B/C ratio), and the internal rate of return (IRR).

Criteria were estimated for each of the two scenarios separately.

Sensitivity analyses were carried out to assess how investment criteria changed with changes in the value of a range of assumptions, including adoption level, probability of development and commercialization, and the reduction in the fungicide and chemical costs after the disease-resistant type is developed.

In the case of the "earlier impact" approach, the base assumption was that BIP caused the impact to occur two years earlier than without the BIP. However, a sensitivity analysis was carried out on the length of this time period.

Attribution of Benefits to BIP

Attribution of benefits to BIP are made on the basis of the present value of costs of BIP in relation to the present value of other funding available from 1994 to 1998, as well as that required from 1998 onwards to achieve the outcomes specified. It is assumed that 30 scientist-years/ year have been contributed outside of BIP over the period 1994-98. In addition, it is assumed that a further 35 scientist-years/year will need to be committed between 1998 and 2012 for the two scenarios specified in the assumptions table to be achieved. Valued at US\$180,000 per scientist-year (full cost), this assumption means that the investment in the period 1998-2012 will be about US\$6.3 million/year, and will include both private and public sector investments.

Results Using the Attribution Method

Results for Scenario 1 – The Transgenic Cavendish. The investment criteria estimated using the base assumptions for scenario 1 are given in Table 4.2.

Even with the conservative assumptions used, there is an expected positive NPV from the BIP investment of US\$27 million, with a B/ C ratio of 10:1. This is despite the time lag assumed of 18 years between the commencement of BIP and the introduction of a disease-resistant Cavendish banana. The US\$27.1 million

Table 4.1 Assumptions used in investment analysis for BIP

Item

Without an R&D Program

this out all hab thogan	
Annual banana exports (million t) Banana yield (t/ha) Export banana production costs (US\$/t) Cost of fungicides (US\$/ha/year) Cost of nematicides (\$/ha/year) Yield loss due to black Sigatoka with efficient chemical control (%) Yield loss due to nematodes with efficient chemical control (%) Yield loss due to nematodes with efficient chemical control (%) Annual increase in cost of fungicide (% /year) Annual increase in cost of nematicide (% /year)	12.6 ^a 40 ^b 169 ^c 1,150 ^d 225 ^d 10.0 ^d 17.5 ^d 1 ^e 1 ⁹
With an R&D Program	
Resource cost of BIP (US\$ million/year) (1994-1998) Other resources devoted to banana improvement worldwide (scientist years/year) (1994-1998) Other resources devoted to banana improvement worldwide (scientist years/year) (1999-2012) Resource cost of a scientist year (US\$/year)	0.7 ^f 30 ^g 35° 180,000 ^h
Outcome: Scenario One (Transgenic Cavendish)	
Year in which transgenic-disease resistant Cavendish commercialized and adopted by growers Probability of development and commercialization of transgenic Cavendish (%) Reduction in cost of fungicides used per hectare after transgenic Cavendish introduced (%) Reduction in yield loss due to black Sigatoka after transgenic Cavendish introduced (%) Reduction in cost of nematicides after transgenic Cavendish introduced (%) Reduction in yield loss due to nematodes after transgenic Cavendish introduced (%) Reduction in yield loss due to nematodes after transgenic Cavendish introduced (%) Reduction in yield loss due to nematodes after transgenic Cavendish introduced (%) Maximum adoption level of transgenic Cavendish (%)	2012 ⁱ 50 ⁱ 60 ⁱ 30 ⁱ 30 ⁱ 80 ⁱ
Outcome: Scenario Two (Alternative Banana Type)	
Year in which new disease-resistant banana type commercialized and adopted by growers Probability of development and commercialization of new disease-resistant banana type (%) Reduction in cost of fungicides used per hectare after new banana type introduced (%) Reduction in yield loss due to black Sigatoka after new banana type introduced (%) Reduction in cost of nematicides after new banana type introduced (%) Reduction in yield loss due to nematodes after new banana type introduced (%) Reduction in yield loss due to nematodes after new banana type introduced (%) Reduction in yield loss due to nematodes after new banana type introduced (%) Cost to export banana industry of changing banana type (US\$ million)	2012 ⁱ 50 ⁱ 80 ⁱ 50 ⁱ 50 ⁱ 50 ⁱ 100 ⁱ

a. CIRAD (1998).

b. Estimated from CIRAD statistics for major exporting countries.

c. Estimated from figures supplied by CORBANA, Costa Rica.

d. Mid-point of range supplied by CORBANA, Costs Rica.

e. Estimate by Agtrans Research.

f. Estimate of annual investment by BIP.

g. Estimate by Agtrans after discussions with CIRAD.

h. Estimate by Agtrans after discussions with Scientific Advisory Panel.

i. Estimate by Agtrans and Scientific Advisory Panel.

j. Estimate by Scientific Advisory Panel; this cost will depend on what level of existing banana production will need to be plowed out due to the changeover, the degree to which the new banana type resembles Cavendish for post- production activities, and whether, and to what degree, market research and promotion is required.

Table 4.2 Expected investment criteria for scenario 1 (transgenic Cavendish) (discount rate 7%)

	NPV (US\$ million)	B/C Ratio	IRR (%)
Total investment	578	9:1	21.4
BIP investment only	27.1	10:1	21.9

Source: Author's calculations.

NPV for the BIP investment could be larger if a higher level of benefits from the transgenic Cavendish is attributed to BIP. The leverage of additional resources into banana improvement as a result of BIP has not been included in the current attribution; in addition, the influence of BIP on the degree of interest from the private sector has been significant, but the implications of this influence have not been included in the attribution of benefits made to BIP.

The estimation of benefits is conservative in two ways. First, the actual parameters used in the quantitative analysis could be considered conservative. Second, there are a number of other benefits outlined in earlier sections that have not been included in the quantitative analysis. Environmental and human health benefits are benefits that are usually difficult to value, but are likely to be high and significant. Such benefits could also provide justification for public investment in banana improvement, since benefits could be captured by communities as opposed to the private sector in bananaexporting countries.

The sensitivity of investment criteria to small chemical cost increases, likely due to larger amounts of chemicals applied and the need to continually develop new products to overcome chemical resistance, is evident in Table 4.3. Although the influence of the rate of increase of this cost is significant, it would have been even greater if not for the lag between the BIP investment and the likely introduction of a disease-resistant variety. Until the year 2012, the cost increases are assumed to apply in both the "with" and "without" R&D situations, so there

Table 4.3 Sensitivity of investment criteriato rate of chemical cost increase

Rate of increase (% p.a.)	NPV (US\$ million)	B/C Ratio
1 (base)	27.1	10:1
2	31.3	11:1
3	36.4	13:1
5	50.5	17:1
10	125.5	42:1

Source: Author's calculations.

is no benefit to the R&D until the year 2012. It is only after the year 2012 that a difference is apparent.

The investment criteria are highly sensitive to the probability of success of the R&D (Table 4.4). The base level probability assumed was 0.5. The break-even probability (where the BIP investment would break even at a 7 percent discount rate) was 0.05, indicating that the chances of development and commercialization do not have to be very high for the investment to provide a worthwhile expected payoff.

The maximum level of adoption of the transgenic Cavendish needs to be above about 8 percent before the investment will break even (Table 4.5). Because the changeover costs of adopting a disease-resistant Cavendish are likely to be minimal, since the changeover could be phased in over a period, it is likely that the adoption level among banana-exporting producers will be quite high. This is reflected in the base assumption used which was 80 percent adoption.

Table 4.4 Sensitivity of investment criteriato probability of success

Probability of success by 2012	NPV (US\$ million)	B/C Ratio
0.10	3.0	2:1
0.25	12.0	5:1
0.50 (base)	27.1	10:1
0.75	42.2	15:1
1.00	57.3	20:1

Level of adoption (%)	NPV (US\$ million)	B/C Ratio
10	0.7	1.2:1
25	6.4	3:1
50	15.8	6:1
80 (base)	27.1	10:1
100	34.7	12:1

Table 4.5 Sensitivity of investment criteriato level of adoption

ladie 4.7	Sensitivity of investment crit	eria
to reduce	ed yield losses	

Reduction in level of yield loss (%)	NPV (US\$ million)	B/C Ratio	
0	15.3	6:1	
10	19.4	7:1	
30 (base)	27.1	10:1	
60	37.8	13:1	
100	50.5	17:1	

Source: Author's calculations.

The level of reduction of chemicals due to the use of a transgenic Cavendish is difficult to assess, without making further assumptions about the source of resistance and the mechanisms involved in conferring such resistance. It is assumed that at least some chemicals will still need to be used. The base level reduction was assumed to be 60 percent of what the use would have been without the transgenic Cavendish. The investment criteria were very sensitive to changes in this assumption (Table 4.6). The NPV is still positive even if there is no reduction in chemical usage, due to the assumption that yield losses will still be reduced.

Investment criteria changed significantly when the assumption concerning the reduction in yield loss due to the transgenic Cavendish was varied (Table 4.7).

Results for Scenario 2–The Alternative Banana Type. The investment criteria estimated using the base assumptions for scenario 2 are given in Table 4.8.

Table 4.6 Sensitivity of investment criteria to level of reduced chemical usage

Reduction in chemical usage	NPV (US\$ million)	B/C Ratio
0	8.7	4:1
10	11.8	5:1
30	17.9	7:1
60 (base)	27.1	10:1
100	39.4	14:1

Source: Author's calculations.

Source: Author's calculations.

With the conservative assumptions used, there is a highly positive expected rate of return from BIP (Table 4.8), with a B/C Ratio of 8:1. The main difference between this future possibility and the transgenic Cavendish outcome is that scenario 2 assumes that there is a cost to the industry of changing the banana type away from Cavendish. The magnitude of this cost is difficult to estimate as it will depend, in part, on the similarity of the new export banana type to Cavendish. Depending on the market development strategy and how quickly change is accommodated, there may be some replanting costs (additional replanting costs to what would have been the case with continuing with Cavendish). But handling, storage, and shipping facilities may also need to be altered and the associated costs could be significant. Although the base assumption is a cost of US\$100 million, this variable is subject to a sensitivity analysisTable 4.9). The break-even changeover cost that could be sustained and still provide a

Table 4.8 Expected investment criteria for scenario 2 (Alternative Banana Type) (discount rate 7%)

· · · · · · · · · · · · · · · · · · ·	NPV	B/C	IRR
	(US\$ million)	Ratio	(%)
Total investment	484	8:1	20.0
BIP investment only	22.8	8:1	20.5

Table 4.9 Sensitivity of investment criteriato changeover cost

Changeover cost (US\$ million)	NPV (US\$ million)	B/C Ratio	
100 (base)	22.8	8:1	
500	17.2	7:1	
1000	10.3	4:1	
2000	-3.5	-0.14:1	

Source: Author's calculations.

7 percent return on R&D investment was estimated at US\$1.75 billion.

The other two major differences between scenarios 1 and 2 were:

1. The maximum adoption level was assumed to be lower for scenario 2.

2. The reduction in chemical costs and yield losses was assumed to be higher in scenario 2.

Most other sensitivity analyses carried out for the new banana-type scenario showed similar trends to those for scenario 1.

The analyses have assumed that either one or the other of scenarios 1 and 2 could occur. Although it is possible that scenarios 1 and 2 could occur, to assess the combined effect would require recasting the assumptions regarding adoption rates.

Results Using the Earlier Impact Method

Results for Scenario 1-The Transgenic Cavendish. Results for the investment analysis using the "earlier impact" method and assuming an earlier impact of BIP of two years (scenario 1

Table 4.10 Expected investment criteria for scenario 1 (Transgenic Cavendish) (discount rate 7%)

	NPV	B/C	IRR
	(US\$ million)	Ratio	(%)
BIP investment	120	40:1	33

Source: Author's calculations.

Table 4.11 Sensitivity analysis of investment criteria to time impact of BIP

Difference in impact timing (years)	NPV (US\$ million)	B/C Ratio	
1	61	21:1	
2 (base)	120	40:1	
3	175	58:1	
5	275	90:1	

Source: Author's calculations.

occurring in 2012 instead of 2014) are given in Table 4.10.

A sensitivity analysis of investment criteria to the time period for the earlier impact is given in Table 4.11.

Results for Scenario 2–The Alternative Banana Type. Results for the investment analysis for the "earlier impact" method for the alternative banana-type scenario are given in Table 4.12. This result is based on an assumption of a two-year speeding up of the realization of benefits.

Given the assumptions made in the analysis, the results reported here suggest that BIP could be expected to provide a rate of return on funds invested of over 20 percent. Conservative assumptions have been used throughout the analyses, and the sensitivity analyses and breakeven parameters estimated show that the results are robust across a credible range of assumptions.

The benefits estimated from this analysis are only a part of the total benefits that would accrue to the investment in banana improvement and, in this regard, the results are likely to be an underestimate of the performance of the investment.

Table 4.12 Expected investment criteria for scenario 2 (Alternative Banana Type) (discount rate 7%)

<u> </u>	NPV	B/C	IRR
	(US\$ million)	Ratio	(%)
BIP investment	104	35:1	31

The attribution method of analysis gave a lower expected rate of return than the "earlier impact" approach, even when the impact of the transgenic Cavendish or the development of the alternative banana-type was assumed only one year earlier (2012 instead of 2013) due to BIP. This is not surprising, since under the attribution method, BIP's share of the benefits from the total investment was less than five percent whereas with the earlier impact method the full benefits of the total investment for two years were credited to the BIP investment.

Conclusion

BIP has provided the methods for transformation and regeneration of bananas, and has moved the world banana industry closer to the reality of a transgenic banana. Transformation and regeneration has now been accomplished. There has also been significant progress demonstrated in terms of new germplasm for use in breeding programs, and methods to make future breeding programs more effective. The most important output from BIP was strategic knowledge that can be used in future R&D projects.

Short-term and long-term benefits, and both content and process benefits, are likely to be derived from BIP. The long-term benefits include those related to reduced costs of production, improved environment, and reduced health risks for workers. Some of the economic benefits were quantified and used as input to a benefit-cost analysis of the BIP investment.

The results of the investment analyses demonstrate that the expected payoffs from development of disease-resistant banana can justify a large investment in banana improvement research. Assumptions were generally conservative and only a limited set of benefits were included in the analysis. Based on the various assumptions used, the expected rate of return was estimated at 20 to 33 percent, the net present value at US\$22-120 million, and the benefit to cost ratio at 8:1-40:1.

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5

Next Steps

BioBanana: International Banana Biotechnology Program

The World Bank has invited participation by all interested parties in an International Banana Biotechnology Program (*BioBanana*), the purpose of which is to jointly fund research to develop biotechnology-based solutions to the major disease problems affecting banana and plantain production worldwide. The problems are, in order of priority, black Sigatoka, nematodes, *Fusarium* wilt, and virus diseases.

The Program will provide the means by which a group of interested parties will address key disease problems, and evaluate and direct the R&D to combat them. These currently intractable problems affect all producers and exporters worldwide, but are beyond the economic and scientific scope of any one country, organization, or company to solve alone. A copy of the complete BioBanana business plan is available from the Rural Development Division of the World Bank. Novel solutions developed through the application of biotechnology will benefit both export production and subsistence food crop production of banana and plantain.

Rationale

Production

Banana is one of the world's top five internationally traded tropical commodities, with a total annual export value of US\$5.3 billion. Total world exports of banana in 1995 were approximately 10.6 million metric tons from 32 countries (FAO Yearbook 1996). Export dessert bananas represent about 10 percent of total world production of banana and plantain. The largest markets are the United States (28 percent of market share) and the European Community (27 percent). The volume of export bananas has increased considerably over the past 25 years, and there is a growing market especially in Eastern Europe, the former USSR, the Far East, and nonproducing countries in Latin America. Most banana and plantain are consumed as a staple food throughout the humid tropics of Africa, Asia, and Latin America.

Disease Control

The export banana is susceptible to increasingly virulent pest and disease problems because of its narrow genetic base. Almost all the internationally traded dessert bananas are the single Cavendish variety. Unlike other major commodities, there has been little success in breeding for disease resistance using conventional methods. There is no commercially acceptable, black Sigatoka-resistant variety available as a replacement for Cavendish, despite some 40 years of effort in conventional breeding. The main threat to the export banana industry is black Sigatoka disease.

Chemical Control

The lack of success in conventional breeding has led to dependence on chemical interventions, which are becoming:

- Less acceptable, as producers and consumers become increasingly concerned about the health and environmental risks associated with pesticide use
- Less viable, with nematicides likely to be banned within five years in several countries
- Less effective, with the evolution of fungicide-resistant strains of black Sigatoka
- More expensive, with up to 40 annual spray cycles required for effective control costing over US\$1000/hectare. The total yearly cost to the export industry is US\$200 million.

Threats

 Black Sigatoka disease: due to the health and environmental risks associated with the increasing cost and frequency of fungicide applications required to maintain satisfactory control; the emergence of fungicideresistant strains of the pathogen; and the lack of potential new fungicides

- Nematodes: due to the toxic nature of the chemicals presently used for control, and which are likely to be banned within the next five years
- *Fusarium* wilt: due to the emergence of strains of the pathogen in Asia able to attack previously resistant Cavendish varieties in the tropics
- Virus diseases: such as BBTV, which causes serious yield losses in some countries and newer viruses able to spread worldwide as tissue cultured planting material.

Progress Toward Improved Disease Control

Recent research has demonstrated that the application of new biotechnologies to banana enable novel genes to be inserted and expressed. These results suggest that further rapid progress is possible to identify, introduce, and evaluate new genes for disease resistance in banana. The key developments to date are given in Box 1.

Conventional breeding approaches have not been successful in generating disease-resistant dessert bananas of export quality. The export trade is dominated by the Cavendish variety, which is grown both by national producers, mainly in Latin America and the Caribbean (about 80 percent of total world exports: Ecuador 30 percent; Honduras 5 percent; Guatemala 8 percent; Panama 8 percent; Colombia 12 percent; and Costa Rica 15 percent), and on plantations owned by international trading companies. Cavendish is resistant to the races of Fusarium wilt presently occurring in the major exporting countries in Central and South America, but not to the new races occurring in the subtropics and in parts of Southeast Asia. Cavendish is also highly susceptible to black Sigatoka disease.

Conventional breeding (at EMBRAPA, Brazil; FHIA, Honduras; IITA, Nigeria and Uganda; CIRAD, Guadeloupe and Cameroon) has had some success in breeding for disease resistance in banana and plantain, generating new,

Box 1 Key technology developments in banana biotechnology

Transformation technologies:

- Reproducible transformation systems developed as part of a "molecular tool kit"
- Transformed plants available for greenhouse and field evaluation of virus resistance

Germplasm collection and evaluation:

· New germplasm identified in India and Vietnam

Breeding for pest and disease control:

- Black Sigatoka (black leaf streak) resistance
 - Sources of resistance in diploids identified by CIRAD
 - Potential new varieties in Brazil and Honduras with Sigatoka resistance
- Fusarium wilt (Panama disease):
 - Tropical Race 4 Fusarium is distributed in the equatorial belt in Indonesia and Malaysia, and is absent from Vietnam
- Nematodes:
 - Musa genotypes have been identified at FHIA, Honduras, with a high degree of resistance to nematodes

Note: Initial outcomes from BIP-sponsored research.

Sigatoka-resistant varieties that are used mainly for subsistence production in Africa.

The Way Ahead

Genes for Sigatoka resistance do exist in the Musa genome (the genus of banana and plantain). If they could be isolated and introduced into one or more varieties that also have the desirable fruit quality attributes for export, using the new techniques of biotechnology, this would be beneficial to all concerned. The banana trading companies and consumers would have high quality fruit, and producers (both small farmers and large plantations) would be able to grow these with substantially less pesticide. The banana-producing countries would have less detrimental pesticide damage to their environment, and farm workers and smallholder producers would suffer less health damage from their exposure to pesticides.

In 1994, the World Bank initiated an internationally competitive research program to mobilize the best available expertise worldwide to address the problems facing banana production. BIP was cosponsored by Common CFC, FAO, and the World Bank. It was managed by the World Bank on behalf of the cosponsors. CFC provided US\$3.5 million over five years. These funds have leveraged substantial other investments in research from both national and international sources. The project and its outcomes are summarized in earlier Chapters.

The results from the research supported through BIP have demonstrated that well targeted research can address some of the industry's problems. The need now is to devise a mechanism to support further strategic research on the applications of biotechnology to banana and plantain. A delivery mechanism also needs to be designed to convert these discoveries into potentially useful products, for both the export banana industry and subsistence production of banana and plantain.

Most of the R&D on banana breeding and biotechnology has been funded through the public sector in producing countries and by development agencies. Because the banana export industry will benefit from the application of newly available biotechnologies, it would be desirable to have a mechanism by which the export industry, through both national and international companies and producer cooperatives, could join with public sector agencies and development agencies in financing the next phase of the research, development, and delivery of new technologies.

The International Banana Biotechnology Program aims to provide a vehicle by which this could be achieved in a cost-effective manner.

Why the World Bank is Involved

The World Bank has invested about US\$25 million in the banana industry in producing countries. This includes substantial investments by the International Finance Corporation (IFC), the Bank's private sector investment affiliate, related to improving the efficiency and the sustainability of the banana industry in several countries of Latin America and the Caribbean and Southeast Asia, through support for production, shipping, and R&D. The World Bank has a vital interest in assisting in the control of diseases on this important crop, reducing environmental damage, and improving worker safety.

The Bank is also supporting R&D on banana and plantain through BIP, and through its sponsorship of the Consultative Group on International Agricultural Research (CGIAR). Two of the CGIAR centers (International Institute of Tropical Agriculture IITA, Nigeria, and the International Plant Genetics Resources Institute, IPGRI, and its International Network for the Improvement of Banana and Plantain, INIBAP) undertake R&D on banana and plantain.

In addition, INIBAP and the World Bank have played a leading role in launching the concept of *ProMusa*, a global program designed to improve synergies among research teams working on banana and plantain worldwide.

The World Bank is exploring ways to work more synergistically and innovatively with the private sector in areas of mutual interest, including in biotechnology, where this can benefit the Bank's developing member countries. The Bank is also examining the future needs and opportunities for developing countries to make safe and effective use of the applications of modern biotechnology to solve previously intractable problems that affect environmentally sustainable agricultural production. It is also reviewing the appropriate policies and practices on intellectual property management to enable developing countries to access and utilize the products and processes of modern biotechnology (Lele, Lesser, and Horstkotte-Wesseler 1999). The Bank is also reviewing the needs and opportunities to reduce pesticide use through the wider application of integrated pest management approaches.

In initiating and managing the proposed Program, the World Bank intends to:

- Capitalize on the results that are emerging to date, which demonstrate that biotechnology could offer solutions to intractable disease problems
- Provide a forum for the interested parties to come together, under the aegis of the World Bank, to address common problems that are beyond the scope of any one company, country, or research institution to solve
- Demonstrate new modalities of cooperation between the public and private sector by implementing a pilot project in the application of biotechnology to solve agricultural and environmental problems in Bank member countries.

Outcomes and Benefits

Outcomes

The intended outcomes of a successful R&D program are:

• Improved health and safety of farmers and other workers

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- More cost effective control of black Sigatoka disease
- Less environmental degradation due to reduced use of pesticides
- Clarification of the threat posed to Cavendish plantings in the tropics by new races of *Fusarium* wilt
- Identification of new varieties of dessert bananas for export that combine the market advantages of Cavendish types with disease resistance.

Benefits

The potential beneficiaries of the research supported by the Program are:

- The participants of the Program who acquire new technologies
- Banana growers in producing countries. Approximately 60 percent of all export bananas are grown by national producers, mainly in Latin America and the Caribbean
- Subsistence farmers worldwide who grow banana and plantain for domestic consumption. The identification of novel sources of disease resistance would be beneficial to all producers, since this resistance could be introduced into banana and plantain varieties favored for local production, as well as into export quality bananas. The CGIAR Centers would be an important vehicle to ensure this spillover of technology occurs to subsistence producers
- Farmers and farm workers in the bananaproducing countries who are at risk from the wide-scale use of pesticides
- The broader community in banana-producing countries, where there would be a reduction in environmental damage if pesticide use could be substantially reduced
- Economic benefits in banana-producing countries resulting from reduced imports of chemical pesticides
- Consumers worldwide, who would be able to purchase quality fruit, grown with

less or no pesticide, similar to organic produce.

Objectives

The objectives of the R&D program to be supported by the Program are, in order of priority:

1. Sigatoka control (40 percent of research budget)

2. To develop more sustainable means for the control of black Sigatoka disease, which will result in a reduction in the frequency and volume of spray applications necessary to maintain economically effective control

3. Nematode control (20 percent of research budget)

4. To develop improved means of control of nematodes, with the objective of reducing nematicide application on banana

5. Fusarium wilt control (12 percent of research budget)

6. To develop improved means for the control of *Fusarium* wilt, by identifying the range of variation in the *Fusarium* wilt pathogen, and its distribution, and to identify sources of resistance to the tropical races of *Fusarium* wilt that presently attack Cavendish varieties in tropical environments in Southeast Asia

7. Virus control (8 percent of research budget)

8. To develop improved means for the control of the important virus diseases, by the identification of novel sources of resistance and introduce these into Cavendish varieties.

Relative Priorities

The first priority of the Program is the improved control of black Sigatoka disease. Commissioning of research on this part of the Program would start as soon as the Program gets underway. Activities on the control of nematodes and other diseases would commence as additional resources become available. The research strategy outlined below will be implemented with this priority in view.

Research Strategy

The aim of the Program is to develop improved means for disease control, primarily through the use of transgenic varieties of dessert bananas of export quality that:

- Have stable resistance to the major banana diseases
- Are acceptable to regulatory bodies both in the producing countries and in the importing countries
- Are ready for glasshouse and field trials within the duration of the project.

Five priority areas for research have been identified to achieve these objectives:

- Genetic analysis of host and pathogens
- Identification and isolation of candidate resistance genes and the characterization and testing of these genes in banana
- Refinement of the enabling technologies for efficient transformation and transgene expression in banana
- Evaluation of the efficacy and stability of the transgenic disease resistance
- Evaluation of IPM strategies incorporating the use of transgenic varieties and/or biological control agents.

Governance and Organization

Membership in the Program would be open to any company, person, or entity engaged in the production, distribution, or marketing of banana or plantain, or the control of diseases of bananas and plantains, and any organization that is financing or intends to finance (through the Program or otherwise) activities in support of banana and plantain.

The members of the Program may include (a) public and private agencies in banana exporting and producing countries, including farmer cooperatives; (b) national and international banana producing and trading companies and other commercial companies; and (c) development agencies with interests in the social and economic development in banana and plantain producing countries.

Council

The Program would operate through a Council that would consist of a representative of each participant in the Program. The Council would:

- Review the strategy and policies of the Program
- Review the progress of the Program, including progress of individual research activities financed by the Program, and the financial situation of the Program
- Consider and approve the annual work program and financial plan and budget for the next year
- Advise the Bank on the appointment of members of the Scientific Advisory Panel.

Program Executing Agency

The World Bank would act as the Program Executing Agency on behalf of the members of the Program. The Bank would carry out the Program through research grants awarded to research providers on the basis of international competition. The Bank would be responsible for the technical management of the Program and the financial management of the contributions to the Trust Fund. The Bank would report to the Council on the technical and financial management of the Program, on a biannual basis.

Scientific Advisory Panel

The Bank and the Council would be advised by an independent Scientific Advisory Panel on the technical content of the research projects supported by the Program.. The major tasks of the Panel shall be to:

- 60 Banana, Breeding, and Biotechnology
- Provide technical advice to the Bank and the Council on the Program, its strategy, priorities and approach to R&D
- Advise the Bank on the selection of the research proposals to be funded
- Assist the Bank in monitoring the progress of the contracted R&D projects against the agreed milestones
- Advise the Bank on the acquisition and disposition of intellectual property
- Assess the impact of the Work Program on an ongoing basis, by assessing the impact of selected activities.

Internationally Competitive R&D Grants

The Program's research activities would be based on an internationally competitive research grants scheme, similar to that used in the Banana Improvement Project. After the formation of the Program, it would be advertised and research proposals would be sought worldwide against the priorities, research strategy, and approach outlined earlier. The Scientific Advisory Panel would advise on the selection of proposals to be funded by the Program that would contribute to the achievement of the objectives of the Program.

Trust Fund

The Program's operations would be financed by contributions from participants to a Trust Fund managed by the World Bank in accordance with the provisions of the Agreement between the Bank and the participants in the Program. The contributions would be renewed on an annual basis for five years, subject to satisfactory progress in the research program. The proposed allocation to the Trust Fund to achieve the R&D objectives is US\$5 million per year (1998 dollars). This represents approximately 2.5 percent of the present annual cost of pesticide applications on bananas (about US\$200 million).

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Appendix 1

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