BILATERAL

# MEMORANDUM OF UNDERSTANDING BETWEEN THE GOVERNMENT OF THE REPUBLIC OF THE PHILIPPINES AND THE GOVERNMENT OF AUSTRALIA RELATING TO THE CONTROL OF RIPENING IN PAPAYA AND MANGO BY ...

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## 1. GENERAL

1.1. This MEMORANDUM expresses the understandings of the Government of the Republic of the Philippines and the Government of Australia concerning the responsibilities and contributions respectively of the two Governments with regard to the implementation of the ACIAR Project No. PN 9445 - Control of Ripening in Papaya and Mango by Genetic Engineering.

1.2. The project aims to improve the postharvest characteristics of papaya fruits. The project will also provide the foundations for plant tissue culture and transformation in mango, one of the more important fruit commodities in the region. The project is further described in Attachment 1 of this Memorandum of Understanding.

## 2. DEFINITIONS

In This Memorandum:

"Australian institutions, firms and organizations" means Australian institutions, firms or organizations engaged in the project;

"Australian personnel" means Australian nationals or permanent residents or other persons who are not nationals or permanent residents of the Philippines, who are working in the Philippines on the project and whose salaries or other costs are funded from the contribution of the Government of Australia to the project;

"dependant/dependent" means the spouse or unmarried minor children of a member of the Australian personnel or any person recognized by the two Governments as dependant/dependent of a member of the Australian personnel;

"professional and technical material" means equipment, motor vehicles, and other goods imported by members of the Australia personnel or Australian institutions, firms and organizations of their professional use in carrying out their assigned duties under the project and paid for from funds provided by the Government of Australia;

"project supplies" means equipment, material and other goods supplied for the execution of the project, the cost of which is funded from the contribution of the Government of Australia to the project;

"services" means services performed by individuals or by general professional partnerships registered in the Philippines as detailed in Attachment 1;

"personal and household effects" means equipment, motor vehicles and other goods imported by Australian personnel or Australian institutions, firms and organizations for the persona) use of Australian personnel and their dependents while engaged in the project;



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## 3. COORDINATING AUTHORITIES AND IMPLEMENTING AGENCIES

3.1. Each Government will implement the research project through respective Coordinating authorities.

3.2. The Department of Foreign Affairs, hereinafter referred to as the DFA, will be the Philippine Coordinating Authority. The Australian Centre for International Agricultural Research, hereinafter referred to as ACIAR, will be the Australian Coordinating Authority.

3.3. Under the terms of this Memorandum of Understanding, the DFA nominates the University of the Philippines at Los Banos, hereinafter referred to as UPLB, to carry out the project on its behalf, and ACIAR nominates the University of Queensland, hereinafter referred to as UQ, to carry out the project on its behalf, as described and detailed in Attachment 1.

## 4. CONTRIBUTIONS

4.1. The contributions of the Government of the Republic of the Philippines and the Government of Australia as described in Attachment 1 are estimated to value One Hundred Thousand, Five Hundred Dollars (AUD$100,500) and Three Hundred and Sixty Three Thousand Dollars (AUD$363,000) respectively. The financial contribution of the Government of the Republic of the Philippines will be subject to its annual Congressional appropriations. The financial contribution of the Government of Australia will be subject to the normal annual parliamentary approval of appropriations.

4.2. Further to the estimated contributions above, additional funds will be provided by the Government of the Republic of the Philippines for payment of taxes incurred through implementation of the project.

## 5. PERSONNEL

5.1. The Government of the Republic of the Philippines and the Government of Australia will provide personnel possessing the competence required for the project, including Philippine and Australian project leaders. The project leaders will have responsibility for effective implementation in their respective countries.

5.2. Personnel composition in UPLB and UQ are described in Attachment 1.

## 6. IMPLEMENTATION, MONITORING AND REVIEW

6.1. Project implementation will follow the schedule specified in Attachment 1. The project leaders will provide a yearly progress report to ACIAR, copies of which will be forwarded to the DFA.

6.2. The Philippine and Australian project leaders will establish regular consultations on project progress, direction and schedules.

6.3. Philippine project leaders will join the yearly coordination meeting of all project leaders from the three countries involved in the project.

6.4. The project will be reviewed approximately six months prior to its conclusion. The main reviewer(s) (1 or 2) will be an independent acknowledged eminent scientist(s) or any nationality, specifically contracted by ACIAR for the purpose. The reviewers will be joined by the relevant ACIAR program coordinator, and a representative from the DFA during the review process in the Philippines. They will participate in the deliberations.

7. PROJECT SUPPLIES, MOTOR VEHICLES AND PROFESSIONAL AND TECHNICAL MATERIAL AND SERVICES


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7.1. In respect of project supplies, motor vehicles, professional and technical material and services whether to be imported into and/or procured within the Philippines for the purposes of the project, the Government of the Republic of the Philippines will:

(a) be responsible for the payment of import duties, Value Added Tax (VAT), and other duties and taxes imposed in the Philippines and be responsible for inspection fees, storage charges and all other levies, fees and charges;

(b) facilitate the expeditious clearance and release of imported project supplies and motor vehicles including the provision of appropriate customs and wharfage facilities in the port of entry closest to the site of the project; and

(c) unless mutually arranged otherwise, provide expeditious internal transport to the site of the project.

7.2. Project supplies and motor vehicles provided by the Government of Australia for the project will be available for the unrestricted use of the project and will not be withdrawn from that use without the consent of the Government of Australia.

7.3. Motor vehicles provided by the Government of Australia for the purposes of the project will be registered as Official Exempt Vehicles and handed over at the completion of the project to the agency or another project as mutually agreed between ACIAR and UPLB. These vehicles will be under the administrative control of the Philippine project leader for the duration of the project.

8. IMPORTATION OF PERSONAL AND HOUSEHOLD EFFECTS AND MOTOR VEHICLES FOR PERSONAL USE

In respect of the importation into the Philippines of bona fide personal and household effects, including motor vehicles, for the personal use of Australian personnel or their dependants/ dependents, which accompany such persons or arrive within six months from either the date of their first arrival in the Philippines, or the signing of this Memorandum whichever is the later, the Government of the Republic of the Philippines will:

(a) be responsible for the payment of all customs duties, other duties, taxes, levies, and other charges of similar nature; and

(b) expedite their clearance through customs.

9. EXPORT OF PROFESSIONAL AND TECHNICAL MATERIAL AND PERSONAL AND HOUSEHOLD EFFECTS

In respect of the export from the Philippines at the end of the assignment of Australian personnel, of professional and technical material, personal and household effects and motor vehicles for personal use, the Government of the Republic of the Philippines will:

(a) be responsible for the payment of all customs duties, taxes, levies and other charges of similar nature; and

(b) expedite clearance through customs and other export controls.

## 10. LOCAL LAWS AND REGULATIONS

The Philippine Coordinating Authority and the Philippine Implementing Agency(s) will exert their best efforts to inform and keep advised Australian firms, institutions and organizations of local laws and regulations which may concern them in the performance of their duties.


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## 11. CLAIMS

The Government of the Republic of the Philippines will deal with any claim which may be brought by third parties against Australian personnel and Australian firms, institutions and organizations and will hold harmless said Australian personnel and Australian firms, institutions and organizations from civil liability from acts or omissions occurring in the course of the performance of their duties in the execution of the project except where both Governments accept that their acts arise from gross negligence and willful misconduct.

## 12. SECURITY

The Government of the Republic of the Philippines will provide security arrangements for Australian personnel and their dependents and property on project sites and elsewhere when they are involved in official activities in accordance with the present Memorandum. The nature of such security arrangements will be mutually determined by the two Governments.

## 13. AMENDMENTS

Amendments to this Memorandum may be made at any time by written arrangement between the parties. These shall take effect on the date of the latter written notification by the Parties, through diplomatic channels, indicating that the domestic requirements for its entry into force have been complied with.

## 14. DURATION

This Memorandum will take effect from on the date of the latter written notification by the Parties, through diplomatic channels, indicating that the domestic requirements for its entry into force have been complied with. The contribution of the Government of Australia to the Project and all undertakings given will cease on 30 June 2000 or on such other date as may subsequently be arranged between the two Governments.

## 15. ATTACHMENTS

Attachments referred to in and attached to this Memorandum form an integral part of it.

SIGNED at the City of Manila, this 4th.day of November 1997.

FOR GOVERNMENT OF THE
REPUBLIC OF THE PHILIPPINES

Date

FOR THE GOVERNMENT OF
AUSTRALIA

Date

## ATTACHMENT 1

## CONTROL OF RIPENING IN PAPAYA AND MANGO BY GENETIC ENGINEERING

## 1. BACKGROUND

Tropical fruits ripen, deteriorate in appearance and eating quality and succumb to postharvest diseases very rapidly after harvest. Poor postharvest characteristics such as poor flavour development, very short shelf-life, quick softening, easy spoilage, sensitivity to low temperatures (chilling injury) and easy pathogen attack (fungi, etc.), are major constraints to profitability for the domestic market, and to the expansion of existing and new export markets.

Studying the ripening of fruits, they are classified as climacteric or non-climacteric depending upon their patterns of respiration and ethylene synthesis during this process. Climacteric fruits are characterised by an increased respiration rate at an early stage in the ripening process accompanied by autocatalytic ethylene production whereas non-climacteric fruits show a different respiration pattern and display a lack of autocatalytic ethylene synthesis.

Ethylene is one of the simplest organic molecules with biological activity and is the only gaseous hormone known to date. Large losses of fruits and vegetables are incurred annually due to ethylene's effects on plant senescence. In climacteric fruits ethylene controls the rate of ripening. Finding methods to control the ripening process and prevent spoilage is extremely important. Various strategies have been employed to prevent or delay fruit ripening in a reversible way, such as ventilation with air under hypobaric pressures and the application of inhibitors of ethylene action (Yang and Hoffman, 1984). However, these approaches are expensive, often fail to regulate fruit senescence in a satisfactory manner and furthermore, the chemical methods are becoming unacceptable by society. In addition to this, in developing countries there is the added problem of creating the necessary infrastructure close to the growing fields and the transfer of technology to the farmers. This lack of infrastructure makes unpractical many of the approaches used in developed countries.

Biotechnological manipulation of genes controlling ethylene production and other important fruit traits have been proven extremely useful and transgenic fruits with improved postharvest characteristics are already available in the international markets. One very especial advantage of this approach is that once the transgenic fruit is developed, it is the final product by itself. No additional technology transfer is needed and no especial training to fanners is required. In this project, the technology transfer will be directed to specific scientific institutions in order to boost their capacity to apply the techniques to solve additional problems in the same commodity, or to solve similar problems in other commodities.

Biotechnology has been declared as one of the flagship programs in the Philippines bid to attain Newly Industrialising Country Status (NICS) by year 2000. The national biotechnology action plan was approved in 1990 by the Science and Technology Coordinating Committee, a multisectorial consultative group organised by the Department of Science and Technology. In 1994 the strategy and priorities for 1995-2000 were identified and listed as 5 National Needs of which the first two were:

1. Genetically improved strains of microorganisms, plants and animals.

2. Effective postharvest technology and cost-efficient processing of biological products.

This project is addressing both needs. More specific subpriority areas have been identified, one of them being "Export Winners". Within the Export Winners papaya is one of the targeted fresh fruits. Dr. William Padolina, Secretary of the Department of Science and Technology, has personally fully supported the project, furthermore he has committed some start-up funds for the commencement of the project in the Plant Breeding Institute in preparation for the full implementation of the ACIAR project.

The Malaysian government has identified biotechnology as one of the key areas to achieve developed country


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status by the year 2020. As a consequence of this the Malaysian National Council for Scientific Research and Development (MPKSN) has recently created the National Biotechnology Directorate (NBD) to coordinate and promote R&D activities, provide financial support and advise on priority areas within the national grant system (Anon 1994). Malaysia recently hosted two international biotechnology conferences: The Second Symposium on Trends in Biotechnology, Meeting the Challenges of the 21st Century (UPM, Serdang Selangor, April 1994) and The International Symposium on Bioproducts Processing, Technologies for the Tropics (University of Malaysia, Kuala Lumpur, January 1994).

This project originated as a logical continuation of the studies conducted by the Australian project leader in the United States where he was involved in research on the molecular aspects of the ethylene biosynthetic pathway. As a result of his research he cloned and characterised several ACC synthase genes (Botella et al, 1992a, 1992b, 1993, 1995). After his arrival to the University of Queensland Dr. Botella identified several commodities to which his area of expertise could provide significant benefits both at the national and international levels within the South East Asian region. Following consultation with research organisations in several countries, Dr. Mendoza and Dr. Villegas (Institute of Plant Breeding, the Philippines) contacted Dr. Botella with a proposal to apply genetic engineering techniques to the solution of papaya postharvest problems. A similar proposal was made by MARDI (Malaysia), therefore a project combining the needs and expertise of both countries is being developed.

## 2. OBJECTIVES

(A) Papaya Molecular Sub-Program

This sub-program aims to clone and characterise the genes that will be used to genetically engineer the different varieties of papaya plants in order to produce fruits with superior postharvest characteristics. The antisense constructs to be introduced in the plants will also be prepared. Most of the cloning/characterisation work will be carried out at the Plant Genetic Engineering Laboratory (UQ) by Filipino and Malaysian Scientists.

Objective 1: Cloning and identification of ripening-related ACC synthase cDNAs in Filipino, Malaysian and Australian papaya varieties.

This objective will provide the genes that will later be used in preparing the antisense constructs and ultimately the transgenic plants with reduced ethylene production during ripening. The ACC synthase enzyme is encoded by a multigene family. Each member of this family is expressed in very particular tissues at very specific times and therefore each gene play a different role in the development of the plant. In climacteric fruits, there is one gene of the family that plays a major role in the control of the ripening process. It is important to clone only those genes that are important in ripening.

The use of antisense technology for the silencing of the selected genes requires a high degree of homology between the gene used in the antisense construct and the target gene. There is no warranty that the gene active in the Malaysian variety will be effective in suppressing the expression in the Filipino variety, therefore we will clone the ripening-induced ACC synthase genes for each country variety in order to avoid unnecessary risks. Once clones are obtained, they will be sequenced and identified as ACC synthase by homology to other sequences present in the DNA data banks.

The cloning and sequencing will be conducted at UQ by Dr. Laurena for the Filipino variety, Dr. Abu Bakar and Dr Lam for the Malaysian variety and the appointed UQ Scientist.

Objective 2: Study the expression patterns of each gene during papaya fruit ripening.

Once putative genes have been cloned and their identity confirmed, they will be characterised by studying the


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respective expression patterns during fruit ripening. This will unequivocally confirm the cloned genes as being the ones active during ripening in each variety. Expression studies will be conducted in UQ by Dr. Laurena, the UQ appointed scientist and by Dr. Abu Bakar and Dr Lam in MARDI.

Objective 3: Preparation of antisense constructs for each gene.

The selected genes will be used to produce appropriate gene constructs under the control of suitable promoter and terminator sequences. The antisense constructs for the Australian and Filipino varieties will be achieved at UQ by the appointed UQ scientist and Dr. Laurena respectively. The constructs for the Malaysian variety will be developed at MARDI by Dr. Abu Bakar and Dr Lam.

Objective 4: Clone and identify ripening-related ACO cDNAs in Australian varieties.

The ripening-induced ACO genes will be cloned and sequenced by and the appointed UQ scientist in UQ.

Objective 5: Study the expression patterns of each gene during fruit ripening.

The expression patterns of the cloned ACO genes will be determined to ascertain their induction during fruit ripening. UQ appointed scientist will perform the studies at UQ.

Objective 6: Production of antisense constructs for each gene.

A transformation cassette containing the cloned ACO and ACC synthase genes in reverse orientation, regulatory sequences and a selectable marker will be constructed by Dr Abu Bakar and Dr Lam and the appointed UQ scientist at MARDI and UQ.

(B) Papaya Transformation Sub-Program

The transformation subprogram will produce transgenic papaya plants of the Australian, Filipino and Malaysian varieties containing copies of the selected gene constructs in their genomes.

Objective 7: Optimise transformation technology for Malaysian and Filipino papaya cultivars.

The existent transformation procedures for papaya can only be efficiently used for the varieties for which they were developed and will provide a starting point to optimise the transformation of the Filipino and Malaysian varieties. New more efficient methods will be developed by Dr. Mendoza and Dr. Villegas at IPB and Dr. Pillai at MARDI. For the Australian variety, a suitable transformation method has already been developed by Dr. Dale at QUT, (collaborative partner in this project).

Objective 8: Transform papaya plants with antisense constructs.

Plants will be transformed with the different antisense constructs at QUT by the UQ scientist for the Australian variety. Filipino and Malaysian varieties will be transformed by Dr. Mendoza and Dr. Villegas at IPB and Dr. Pillai at MARDI respectively. Transgenic plants will be screened and transformation confirmed by scientist at UQ, IPB and MARDI for the Australian, Filipino and Malaysian varieties respectively. The expression of the antisense transcripts will be analysed in the transgenic plants and those with the highest levels will be selected.

Objective 9: Start glasshouse evaluation of transgenic plants.

The selected transgenic lines will be grown in PC2 containment glasshouses (UQ, MARDI, PBI) under the supervision of the corresponding biotechnology regulatory authorities in each country.

(C) Mango Transformation Sub-Program

Objective 10: Establish mango callus cultures.


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This objective will have two aims. Firstly to develop an understanding of embryo development in mono embryonic and poly embryonic mango seeds. Four cultivars will be studied, two polyembryonic varieties (Kensington and Carabao) and two monoembryonic varieties (Kent and Tommy Atkins). Kensington is the major variety in Australia and Caribao is the most popular variety in the Philippines, being exported in large quantities.

The second aim will be to initiate callus cultures using embryo explants at the most amenable stage of development.

Objective 11: Obtain embryogenesis system for mango cultures.

Once callus cultures have been started in year one, embryogenic cultures will be proliferated and progressed through the various stages of embryogenesis until mature mango embryos have been produced. It is expected that different cultivars will perform differently and that some will be more amenable to embryogenesis and maturation than others.

Objective 12:Optimise mango regeneration.

Those cultivars producing mature embryos will be regenerated and plantlets produced in culture. Subsequently, germinated normal plantlets will be establish in potting mix in a glasshouse.

## 3. METHODOLOGY

The cloning of ripening-related ACC synthase cDNAs will be achieved by reverse transcription coupled to polymerase chain reaction (RT-PCR). Papaya fruits will be collected at the ripening stage in which ACC synthase content is expected to be maximum and total RNA will be extracted. Although no RNA extraction method has been published to date, Dr. Botella has developed a method to extract high quality RNA from papaya fruits. This RNA will be reverse transcribed to obtain a cDNA population enriched in ACC synthase transcripts. The ACC synthase cDNA will then be amplified by PCR using degenerate oligonucleotides designed from highly conserved regions in the ACC synthase protein sequence as primers. Dr. Botella has used the strategy proposed here to clone five ACC synthase genes in mungbean, two in mango fruits, three in pineapple (one in fruits and two in leaves), four in apple and four in geraniums (Botella et al., 1992b; Botella et al 1993; Cavallaro and Botella, 1995; Cazzonelli and Botella 1995 and unpublished results). Dr. Laurena will work at the Plant Genetic Engineering Laboratory (UQ) during the first year of the project to receive in depth training in molecular biology techniques including RNA extraction, RT-PCR, sequencing, cloning, construction of DNA cassettes, etc. He will be in charge of the molecular biology component for the Filipino variety under the direct supervision of Dr. Botella. Dr. Abu Bakar will also receive training in RT-PCR at UQ in order to clone the gene for the Malaysian variety. DNA will be sequenced by the dideoxy chain termination method (Sanger et al, 1977). Sequence analysis and homology searches will be performed using the Blastn and Blastx programs in the Australian National Genomic Information Service (ANGIS).

To conduct expression studies, fruits at five different ripening stages will be selected and total RNA extracted. The expression of the cloned ACC synthase genes will be analysed by northern hybridisation under high stringency conditions. Once the expression is determined the genes will be inserted in reverse orientation under the control of the CaMV 35S promoter and NOS terminator sequences in a suitable plant transformation vector.

ACO genes will be cloned, sequenced, their expression studied and antisense constructs made using similar methods to the ones described above for ACC synthase, (RT-PCR, Northern analysis, etc.). The work for the Malaysian varieties will be conducted in MARDI by Dr. Lam Peng Fatt with a training component at UQ. The cloning and characterisation of ACO gene for the Australian variety will be conducted at UQ.


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Papaya Transformation Sub-program

Gene transfer to papaya has been reported by Agrobacterium-mediated transformation (Pang and Sanford, 1988; Fitch and Manshardt, 1990) and via microprojectile bombardment (Fitch et al., 1990). Due to the higher transformation efficiencies observed, the biolistic gun will be the method of choice. Unfortunately, the transformation methods are not readily interchangeable even among varieties of the same fruit and therefore optimisation of the different parameters will have to be developed for Solo and Eksotika papayas.

Dr. Jim Dale and his research team at QUT have optimised the methods originally reported by Fitch et al. for Australian papaya varieties. As a result, a modified more efficient and faster method is now available (Ballin et al. 1994; Drew et al. 1994). We will use this method to introduce antisense copies of ripening induced-ACC synthase and ACO into the Australian variety. Also, the method developed by Dr. Dale will be taken as starting point to optimise specific transformation and regeneration methods for the Filipino and Malaysian varieties.

An outline of the optimisation process for the Malaysian and Filipino varieties follows. Immature somatic embryos will be excised from green fruit and will be induced to form somatic embryos. Transient expression studies will be performed to determine the optimal conditions to be used for stable transformation. For the transient expression experiments, a standard vector carrying the nptII (kanamycin resistance) and uidA (ß-glucuronidase, GUS) genes under the control of suitable promoter and terminator sequences will be used. Optimal gas pressure and distance to the target tissue will be determined. The effect of osmotic treatment will be determined by culturing the embryos on high osmolarity medium for different times prior and after bombardment. The effect of culturing the embryos in liquid media prior to bombardment will also be investigated. Once the optimal conditions for transient expression are established, they will be used to produce stable transformants. After bombardment, embryos will be allowed to recover for 2-3 days and then' placed in solid medium containing an appropriate concentration of selection agent. After selection, embryos will be transferred to media containing low levels of selection agent and plantlets allowed to develop.

Plantlets will be planted in soil and gradually hardened. When enough tissue is available, molecular characterisation of the transgenic plants will be performed. The presence and copy number of the transgene in each transformed plant will be determined by Southern analysis. The integrity of the transgene will be determined by PCR analysis using primers positioned 5' and 3' of the promoter and terminator regions respectively. Expression studies will be performed by northern blot in order to determine the levels of expression of the antisense gene in the transgenic plants. Those plants with high levels of antisense expression will be selected and grown in containment glasshouses.

Mango Transformation Sub-program

Litz (1984), initiated callus cultures in three of nine monoembryonic cultures of mango. De Wald et al. (1989) extended this protocol to initiate callus cultures from nucellar tissue of two polyembryonic cultivars. However the protocols are cultivar-specific and some varieties are very difficult to establish in vitro. Similarly, somatic embryo production could be induced from these callus cultures, however control of development of mango somatic embryos to maturity took up to 6 months and was difficult to achieve. Physiological abnormalities can develop in this embryos during the long developmental stage. De Wald et al. (1989) developed a number of procedures to overcome these problems for two polyembryonic varieties and regenerated normal plants from somatic embryos.

Development of the embryos in seeds of the four selected cultivars will be studied and the optimum stage of development for callus initiation determined. To achieve this, explants at various stages of maturity will be established in vitro. A range of media and culture conditions will be tested for callus initiation.


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During the second year, tissue culture experiments will be aimed at development and maturation of secondary embryos. A range of media treatments will be tested using the conditions published by Litz (1984) and De Wald et al. (1989) as control. Emphasis will be given to controlled development of embryos to maturity while avoiding abnormal development and precocious germination.

Once mature embryos are obtained they will be germinated in vitro. Initially the procedures of De Wald et al. (1989) will be used. If they are not suitable for the selected cultivars, new media and procedures will be tested. Germinated plantlets will be carefully acclimatised and planted in potting mix in a glasshouse.

## 4. APPLICATION AND BENEFIT OF RESEARCH

Application

The specific application of this research is the development and commercial release of homozygous dominant transgenic lines of papaya with superior postharvest and transport characteristics. These lines will be derived from selected cultivars in general use or acceptable to the traditional Filipino, Malaysian and Australian markets.

In some projects, the transfer of the resulting technology to fanners or regional centres is complicated, other times in order to implement the results it is necessary the development of specific infrastructure. The transfer of technology in our project will be extremely simple. Once the final transgenic lines are developed and approved for release, seeds will be distributed to farmers by official extension services and commercial seed suppliers. No special training to the farmers will be required and no special infrastructure will have to be put in place. Both the EPB and MARDI have developed their own papaya varieties previously and therefore have experience in commercialising new plant varieties.

IPB has developed and released more than 60 superior varieties of 19 crops like high-yielding and disease resistant corn, peanut, soybean and papaya. These are now widely planted by farmers nationwide and used as breeding materials in other countries. The National Seed Foundation within the IPB produces and markets seeds of the new varieties obtained in the institute. IPB maintains linkages with farmers, government and foreign agencies, private companies and non-government organisations in the distribution and dissemination of seeds. It also offers extension services like training, lectures, demonstration farms, technical advisory assistance and information dissemination through library information system, publication and exhibits. Extension research in monitoring the adoption and impact of improved IPB varieties in farming systems nationwide is being implemented.

MARDI has extensive experience in obtaining improved plant varieties. Among them are Eksotika and Eksotika II, two papaya varieties developed in MARDI that have had a big impact in the importance of this commodity in Malaysia. MARDI do not only develop new varieties but also propagate and distribute them to ensure ready access to the farming communities

Genetic manipulation and transformation of papaya is a generic technology and therefore can have generic applications. The transfer of the technology to the research organisations in the Philippines and Malaysia will enable them to apply genetic engineering techniques to the solution of other problems such as production of transgenic cultivars resistant to the papaya ringspot virus. In the same way any other useful genes that could be available in the future could be incorporated in the mentioned varieties.

The optimisation of an embryogenesis and regeneration system for mango also have generic applications. The methods developed here will be used to transfer useful genes to mango. It is important that such a system is established in Australia since it could be used as a basis to optimise the same methods to Asian cultivars. If the technology is available, scientist from other countries could visit UQ and receive training in the basic


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methodology. For commodities such as mango in which breeding is so difficult, genetic manipulation will provide an invaluable tool for variety improvement.

Benefit

Who will benefit from the success of the research?

> Filipino, Malaysian and Australian economies through the provision of long shelf-life papayas leading to decreased spoilage and increased postharvest disease resistance (Appendix 4). This will increase the availability of fruits without the need to increase the yield. The benefits will reach the internal and exports markets.
>
> Filipino, Malaysian and Australian farmers, again through decreased spoilage that will assure maximum return per crop.
>
> Retailers through longer fruit shelf life.
>
> Consumers through the gain of additional time to consume the fruits after purchasing retaining all the quality characteristics.
>
> Filipino and Malaysian researchers through the development of expertise in molecular biology, genetic engineering, tissue culture and plant transformation techniques. These techniques are generic and can be adapted to a wide range of plant species and novel genes.

## 5. STRUCTURE AND COORDINATION OF PROJECT

The project has been divided in distinct subprograms to facilitate coordination. Each subprogram has a nominated leader that will keep continuous contact with Dr. Botella by E-mail. Filipino and Malaysian researchers will have continuous support from researchers in Australia and most of the cloning/tissue culture research will happen in parallel.

Australian Coordination

Australian collaborators will meet every three months at UQ to discuss individual research progress, budget spending and ensure satisfactory progress. They will be kept up to date on overseas collaborators progress and will help to solve any difficulties that might arise in their research tasks. Issues relating to each subprogram will be discussed and coordination between timing of subprogram outputs maintained. Since the Australian collaborators are located in Brisbane, no funds have been budgeted for these meetings.

Overseas Coordination

The Australian project leader will travel to the Philippines and Malaysia twice a year to personally interact with researchers overseas and ensure that adequate progress is

made. Any areas of special difficulty will be identified in these visits and corresponding solutions will be planned and implemented. There will be a mid project workshop during the second year of the project in Brisbane to review Filipino, Malaysian and Australian progress. This review will centre on the cloning of the target genes and tissue culture/transformation methods for the overseas varieties. All the Australian collaborators, two researchers from IPB and two from MARDI will attend the meeting.

End of Project Workshop

The workshop will discuss the technical outputs generated by the project and the immediate plans for the application of the results. Mid and long term applications will also be discussed such as transfer of virus


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resistance to Filipino and Malaysian papaya varieties. New fruit and vegetable crops will be identified as immediate targets for genetic engineering and strategies devised. The workshop will be held in the Philippines to assure maximum local assistance.

## 6. PERSONNEL

University of Queensland and University of the Philippines will appoint or allocate the following staff to this project:

Personnel/position

Percentage time/annum

Dr. Jose Botella

40%

Postdoctoral Scientist

100%

Postdoctoral Scientist

100%

Mr. Tony Cavallaro

20%

Mr. John Bertram

20%

Mrs. Lynn Jessup

20%

Mr. Jos Van der Auwera

30%

Mrs. Sue Bertram

20%

Mr. Dirk Plooy

20%

Mrs. Linda Gust

20%

Laboratory Technical Assistant

100%

Dr. Rod Drew


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10%

Dr. James Dale

5%

Dr. Mae Mendoza

30%

Dr. Tony Laurena

60%

Dr. Violeta Villegas

30%

Mr. Pablito Magdalita

50%

Mr. Reynold Pimentel

50%

Mr. Renato Avenido

50%

Laboratory Technical Assistant

100%

## CORRESPONDENCE

All correspondence relating to this Arrangement should be addressed as follows:

Director
ACIAR
GPOBox 1571
Canberra ACT 2601
AUSTRALIA

SIGNED at.....on the.....day of.....199...

Signature

(Sgd.) RUBEN L. VILLAREAL
Chancellor, U.P. Los Banos

Name and Designation
for the University of the Philippines

Date 2/7/97

Signature

(Sgd.) JAN MASSEY

Director, Research Services
Office of Research & Postgraduate Studies

Name and Designation
for the University of Queensland

Date 12/6/97

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