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Final report

project	**Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers**
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1 Acknowledgments

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2Executive summary

The project *Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers* was initiated in response to declining rice farmer incomes, increased risk to food production and evidence for over-application of fertilisers in paddy rice production, with negative environmental impacts. Although the potential for 'free-living' inoculant microorganisms to enhance plant growth and yield has been known for decades, their production and use in agricultural systems has been limited because of uncertainties about inoculant quality, methods for application, mechanisms of action and the consistency of their performance. It was with foresight that ACIAR and the project participants predicted the need for scientific evidence to facilitate uptake of this rapidly evolving, low-cost solution for more sustainable agriculture.

The key objectives of the project were to optimise, extend and promote biofertiliser technology on rice farms in Vietnam and Australia for increased profit; to develop quality control tools to ensure product efficacy and farmer confidence; to assess the commercial feasibility of biofertiliser manufacture and sale; and to better understand the mechanisms of action of plant-growth promoting (PGP) microorganisms. The main experimental program in SMCN/2002/073, completed by June 2007, yielded a uniquely valuable dataset from more than 20 field experiments showing beneficial effects of the inoculant biofertiliser BioGro. Key conclusions made by the Project Reviewers in October 2007 included:

- A significantly improved understanding of how to maximise the benefits of inoculation, particularly regarding the practicalities of application such as when, how and with how much chemical fertiliser. Nitrogen fertiliser response curves showed that plant growth promotion and improved grain yields could be gained with normal N-fertiliser application plus BioGro, or that BioGro could substitute up to half the applied urea without loss of yield. The benefits flow mainly from the reduced use of urea, associated cost savings and frequent improved rice yields.

- The project developed quality control (QC) methods in order to the identify and quantify the biofertiliser strains used in BioGro. Importantly, the microbial strains selected for BioGro by Professor Nguyen Than Hien identified in Sydney, including *Pseudomonas* and *Bacillus* spp., match those being studied in the global scientific community. This verification that the selection techniques used led to reliable properties in the product is scientifically compelling. A major product of the project is a quality control manual now being published.

- An economic analysis of the field experiments shows that cost savings and yield benefits depend particularly on the rate of biofertiliser application and its cost. However, the potential for national savings in urea fertilizer use is significant (at a rate of adoption of 10% of the rice area 76,000 t urea would be saved); an economic analysis of biofertiliser production shows the potential for widespread village-level employment and other benefits.

An extension of the project from mid-2007 (Variation 2) allowed further data analysis and the effective project review. Following a recommendation by the ACIAR Program Manager Dr Gamini Keerthisinghe, funds to provide more modern basic microbiology equipment for the Biofertilizer Action Research Centre (BARC) were also made available.

This equipment was purchased by the University of Sydney and is functional in BARC's location in Hanoi.

Analysis and publication of data from the experimental program continued in Vietnam and Australia until the major project review was held at the MDI in Can Tho University in October 2007. A final BioGro workshop was held immediately after the Review at the Hanoi College of Science. The presentations of the Workshop were used as the basis of ACIAR Proceedings130, with the text edited by the University of Sydney team and Vietnamese participants and presented to the publisher in June 2008. The Proceedings were published in September 2008 by ACIAR's publication team in time to assist with a bid for a World Bank Global Development Marketplace Innovation Award that would allow implementation of the technology. These Proceedings are available from the ACIAR website, summarising the results of ACIAR's research program in Vietnam in this area since the first small ACIAR project was concluded in 2001.

The success of the World Bank development project DM#5227 *Sustaining nitrogen-efficient rice* led to a revised schedule in Variation 4, ACIAR providing additional funding for research as an aid to optimise the application of BioGro. This development project was developed as a result of this ACIAR project and proposes a franchising or licensing arrangement for supply of high quality BioGro to rice farmers in southern Vietnam, including the Mekong Delta. The model tested was developed as an extension of Objective 3 in the ACIAR project and is a key factor for achieving sustainability of the technology. In addition, ACIAR funding for soil and plant analysis in Variation 4 allowed refinement of schedules for applying BioGro and chemical fertilisers, varying with the season and soil type, to substantially improve site-specific management and the associated economic returns.

The overall impacts of this ACIAR in terms of scientific and rural capacity cannot be understated. The scientific knowledge generated is now internationally accessible through 2 books, 9 peer-reviewed journal articles and numerous presentations. The project enabled the training of over 10 Vietnamese scientific researchers located in numerous institutes throughout Vietnam, along with three doctoral students, two of whom are from developing countries in S.E. Asia. The total number of farmers directly involved in the project and now acting as 'champions' for the use of inoculant biofertilisers numbers over 150, with estimates of over 1000 new farmers having being exposed to the knowledge generated in this project. The most recent data from the participatory field trials indicates that over 80% of the farmers using BioGro increase yields by over 5% and income by an average of 2M VND ($100 USD) per season, relative to the 2010 national average per capita income of $1168 USD (USDS, 2011).

3Background

The majority of Vietnam’s poor live in the north-central and north-east regions, in the Mekong Delta and Central Coast regions: these areas are home to seven out of ten of Vietnam's most economically vulnerable (IFAD, 2011). The Mekong delta represented 21% of the Vietnamese poor in 2004, and is projected to rise to 27% by this decade (AusAID, 2004). Of these people, 90% obtain most their income from agriculture, with 45% of the rural population so poor that they are highly vulnerable to environmental and economic shocks. The major agricultural activity in these regions is rice cropping, with a total cultivated area of more than 1 million hectares (ha) of paddy rice in the Mekong Delta alone and more than 2 million ha country-wide.

Over the last two decades, despite increasing yields, rice farmers’ earnings have been less than expected because of price hikes in materials and labour costs (Vietnam MARD). The price of nitrogen (N)-fertilizers such as urea — usually recommended in Vietnam to be applied at a rate of about 100 kg urea-N per ha — has been among the most affected. Yet no more than 40 kg of N per ha is recovered in the rice crop and the remainder (usually around 60 kg) is lost to the environment directly as ammonia, as nitrate in ground water or is evolved as the greenhouse gas, nitrous oxide. While better management with split applications or deep placement of fertilizers can have some beneficial effects on improving the efficiency of applied N, there is a trade-off in increased labour needs. Overall, more than 30 million Vietnamese involved in rice production are affected economically by this problem of increasing input costs. Any new technology that can improve the use of fertilizers, reducing the total input costs and improving environmental health, would significantly increase the wellbeing of the rural poor in these areas.

Recent research has shown that this problem of high cost and waste of chemical fertilisers can be reduced if steps are taken to improve the efficiency of nutrient-use by rice plants. More efficient uptake of nutrients can be achieved by ensuring that specific microorganisms are present in the root zone of rice plants. These beneficial microorganisms are now widely known as 'plant-growth promoting rhizobacteria', or PGPRs for short, after a series of papers in the late 1970's and early 80's by Kloepper and colleagues (Kloepper and Schroth, 1978; Kloepper et al, 1980). Although these early papers identified the antagonism of deleterious or pathogenic microorganisms as the mode of action of PGPRs, subsequent laboratory and glasshouse research outlined a number of additional mechanisms of action, including:

- fixation of atmospheric nitrogen that is transferred to the plant,
- production of siderophores that chelate iron and make it available to the plant root,
- solubilisation of minerals such as phosphorus,
- synthesis of phytohormones.

One of the first attempts to put together a commercial inoculant biofertiliser comprised of free-living PGPRs was that by Professor Nguyen Thanh Hien, at Hanoi University of Science in the 1990’s. This biofertiliser product, called BioGro, originally contained three strains of bacteria selected from the rhizosphere of rice on the basis of their PGPR activity along with their rapid growth rates and compatibility. The potential of this product to increase rice yields in the field was demonstrated in the north of Vietnam by Professor Hien, Professor Kennedy, and their colleagues, during a small ACIAR project in 2000 and a 2-year project (2001-2) funded by an AusAID CARD grant (Nguyen at al. 2003).

Despite these results, and the results of other studies around the world on PGPRs, the uptake of commercial PGPR biofertilisers by farmers in Vietnam remained limited. The reasons for this were based on a number of valid criticisms, including:

- an overall limited awareness amongst farmers on the availability of biofertilisers and how to use them.
- a lack of quality control on the biofertiliser products, including information on the specific type, mode of action and number of PGPR organisms in a product,
- a lack of scientifically validated field experiments demonstrating consistent performance of a PGPR product under environmentally variable field conditions,
- a lack of confidence in the potential economic benefits afforded by biofertiliser products compared to conventional chemical fertiliser.

Such scepticism about PGPR biofertilisers was (and still is) not limited to farmers in Vietnam, but also to farmers in Australia and elsewhere around the world. However, the chief investigators in this project viewed this scepticism to be similar in nature to that surrounding *Rhizobium* inoculants over 50 years earlier. In order for PGPR biofertilisers to provide economic and environmental benefits to rural communities, and to improve food security in developing countries such as Vietnam, the key issues listed above needed to be satisfactorily addressed, justifying the investment made by ACIAR in this project.

4Objectives

In order to address the key issues raised above, four objectives were originally proposed by this project. These were:

- To conduct field trials in Vietnam and Australia designed to optimise, extend and promote biofertiliser technology on rice farms for increased profit, and to extend this technology to the south of Vietnam
- To design and evaluate a set of simple field tests for quality control of biofertiliser products aimed at ensuring their effectiveness under typical paddy rice field conditions
- To investigate the economic and commercial feasibility of inoculant biofertiliser production in Vietnam
- To conduct laboratory and field research to reveal the mechanisms of the PGPR biofertiliser effect

In preliminary discussions with ACIAR, the first three of these objectives were given greater weight in the use of project resources, given their focus on gaining benefits for farmers. An extension of the project from mid-2007 (Variation 2) allowed further data analysis and the effective project review. This was followed by Variation 3, a technical extension to enable funds to be accessed by the Institute of Agricultural Sciences of Southern Vietnam. A final extension (Variation 4) was granted in mid-2008, after members of this ACIAR project won a World Bank Development Marketplace (WBDM) Award. The WBDM project was conceived as a result of the original ACIAR project and proposed a franchising arrangement to sustainably supply high quality BioGro to rice farmers in southern Vietnam including the Mekong Delta. One of the main goals of this WBDM project was to promote the uptake of biofertiliser technology in southern Vietnam by allowing more than 50 farmers to conduct their own field trials using BioGro, under the supervision and training of the two ACIAR partners, MDI and IAS. In order to value-add to this capacity building project, ACIAR provided additional funds (Variation 4) for improving the agronomic reliability of the biofertiliser technology. The specific objectives of Variation 4 were:

- To correlate soil type and texture with N-use efficiency, with a view to optimising the urea-N level to be recommended with BioGro,
- To quantify the improvement in N- and P-use efficiency as a result of the application of BioGro.
- To optimise the rate of application of BioGro with a view to further reducing farmers' input costs
- To optimise the resource management commercialisation of BioGro production and application

5Methodology

Various methodologies were used to address the project objectives. These methods are described in detail in a number of publications resulting from this work, in particular *ACIAR Proceedings 130: Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers.* A summary of the methodology is outlined below, covering the four original objectives in section 5.1 and the four additional objectives (variation 4) in section 5.2.

5.1 Original Proposal

5.1.1 Objective 1: To conduct field trials in Vietnam and Australia designed to optimise, extend and promote biofertiliser technology on rice farms for increased profit, and to extend this technology to the south of Vietnam

Within objective 1, each co-operating partner organisation was assigned specific tasks in order to tackle the key issues identified as limiting the effective agronomic application of biofertilisers. These issues were addressed scientifically by designing and establishing factorial field experiments, collecting growth, yield and nutrition data, and statistically analysing the results. They included:

The effect of BioGro application rate on rice yield (BARC)

- *Design* Non-replicated farmer field trials at six sites, Spring 2006
- *Factors* Rate of BioGro application: four levels; 0, 50, 100, 200 kg
- *Measurements* Yield components and total yield

The effect of BioGro application timing on rice yield (BARC)

- *Design* Non-replicated farmer field trials at three sites, Spring 2007
- *Factors* Time of BioGro inoculation: four levels; no inoculation, one inoculation (nursery), two inoculations (nursery and transplanting), three inoculations (nursery, transplanting, and tillering)
- *Measurements* Yield components and total yield

The effect of seasonal reinoculation on rice yield (BARC)

- *Design* Randomised complete block design, four replicates, over 3 seasons (starting Spring 2006)
- *Factors* Number of BioGro reinoculations: four levels; no inoculation, one season of inoculation, two seasons of inoculation, three seasons of inoculation
- *Measurements* Yield components and total yield

The effect of BioGro on rice yield and quality of different rice cultivars (VAAS)

- *Design* Split-plot design, with treatments in main plots and varieties in subplots, in triplicate (two seasons, Spring 2006 and Summer 2006)

- *Factors* Fertiliser treatment: Three levels; control (no BioGro plus full fertiliser), BG 1 (BioGro plus half N and P fertiliser), BG 2 (BioGro plus 30% N and P fertiliser) × Cultivar: Six levels (different genotypes)
- *Measurements* Rice yield and rice quality (unhusked grain, % protein, % amylose)

The effect of BioGro and fertiliser interactions on rice yield and quality of two high-quality rice cultivars (VAAS)

- *Design* Randomised complete block design in triplicate (four seasons, Spring 2005 - Summer 2006)
- *Factors* BioGro treatment: Two levels; control (no BioGro) and BioGro × Fertiliser Treament: Five levels; 0, 25%, 50%, 75%, 100% of recommended N and P.
- *Measurements* Rice yield and rice quality (unhusked grain, % protein, % amylose)

The effect of BioGro and N on yield and nutrition of rice (IAS)

- *Design* Split-plot design, with BioGro treatments in main plots and N rates in subplots, replicated 4 times (first and second rainy season 2006)
- *Factors* BioGro treatment: Two levels; control (no BioGro) and BioGro × N Treament: Five levels; 0, 30, 60, 90, 120 kg N ha^{-1}.
- *Measurements* Grain and straw yield and N and P uptake in grain and straw

The effect of BioGro and P on yield and nutrition of rice (IAS)

- *Design* Split-plot design, with BioGro treatments in main plots and P rates in subplots, replicated 4 times (first and second rainy season 2006)
- *Factors* BioGro treatment: Two levels; control (no BioGro) and BioGro × Fertiliser Treament: Four levels; 0, 10, 30, 60 kg P ha^{-1}.
- *Measurements* Grain and straw yield and N and P uptake in grain and straw

The effect of BioGro and other PGPR organisms on the growth, nutrition and yield of Australian rice (USyd)

- *Design* Randomised complete block design, replicated 4 times (first and second rainy season 2006)
- *Factors* PGPR treatment: Five levels; control (no PGPR, 50% N fertiliser), full N fertiliser, BioGro, *Rhizobium sp.*, *Azospirillum* plus *Herbaspirillum*
- *Measurements* Grain and straw yield and N and P uptake in grain and straw

Also within objective 1, a number of field trials were established to assess the economic performance of using BioGro in conjunction with reduced chemical fertiliser application, as compared with the economic performance of farmers' usual (full) chemical fertiliser application.

5.1.2 Objective 2: To design and evaluate a set of simple field tests for quality control of biofertiliser products aimed at ensuring their effectiveness under typical paddy rice field conditions

In order for biofertilisers to be effective and accepted by farmers, their quality needs to be assured, in terms of the number and type of viable organisms per gram of biofertiliser. In this objective, a variety of counting methods were assessed in order to develop a robust and inexpensive quality control protocol for use in biofertiliser production in developing countries. Detailed methodology will be available in the forthcoming manual entitled *Practical methods for the quality control of inoculant biofertilisers* (Deaker et al., 2011), which is currently in press as an ACIAR Monograph. A summary of the methodology is given below.

Identification of biofertiliser strains

- Six PGPR organisms used in the two BioGro formulations were investigated.
- Preliminary identifications were made using conventional microbiological techniques, including morphology, Gram stain, physiology and biochemical characteristics.
- Identifications were confirmed at the University of Sydney by using molecular genetic analysis (16SrDNA for bacteria, ITS region for fungi)

Development of Selective Plate counting enumeration method

- The resistance of each strain to different antibiotics was measured
- Specific antibiotics were incorporated into modified nutrient agar media to allow for the selection and enumeration of individual strains by traditional plate counting

Development of Most-Probably Number (MPN) enumeration method

- The nutritional diversity of each BioGro strain was assessed using commercial API 50 carbohydrate strips (bioMerieux, France). These strips determine the capability of microbial strains to grow on 50 different carbohydrates, giving a positive yellow or a negative red colour in the liquid media
- Using this nutritional information, an MPN method was designed for each strain based on their ability to ferment a single different carbohydrate as the sole C source.

Development of immunoassay based enumeration methods

- Polyclonal antibodies to individual strains were produced in rabbits and the IgG component isolated using affinity chromatography
- The specificity and sensitivity of each IgG fraction was determined by tube agglutination and enzyme-linked immunoassay techniques
- Immunoassays were optimized by examining different IgG dilutions, different extraction techniques from peat media, different growth conditions and calibration methods against pure cultures

Development of immunoblotting enumeration methods

- Immunoblotting methods were optimized by using the IgG developed above. Optimization experiments included the selection of suitable and inexpensive blotting membranes, optimization of binding times and optimization of washing steps.

Development of Quality Control guidelines

- Laboratory trials were conducted to evaluate the cost and performance of each method. Performance parameters included sensitivity (i.e., limit of detection, number of false negatives), specificity (i.e. number of false positives), analysis time and analytical simplicity
- Comparisons were made by using each method to enumerate the BioGro strains in sterile peat, non-sterile peat and soil.

5.1.3 Objective 3: To investigate the economic and commercial feasibility of inoculant biofertiliser production in Vietnam

Three major aspects of the commercial feasibility of biofertiliser production were identified as key to sustainable uptake of biofertiliser technology. These included legal obligations as dictated by government regulation, potential market size as affected by farm level benefits and the actual production models. Methods used to investigate the impact of these on biofertiliser production feasibility included:

Regulation and extension of biofertiliser technology

- The legal definition of a biofertiliser in Vietnam was reviewed (Vietnam Standard TCVN 6169-1996) and the issues arising from the application of biofertiliser in this project were addressed
- These included the microbial composition of the biofertiliser, the biosafety of the strains comprising the biofertiliser, the quality control of the end project and the shelf-life of the end product
- The potential integration of biofertilisers in government-endorsed programs such as the ‘three reductions, three gains’ program were reviewed

BioGro and farm level benefits

- The results from all field experiments conducted by the four Vietnamese institutions participating in this project were compiled, including all input and output data
- Inputs (e.g. fertilisers, labour, fuel, land, seed, plant protection products) and outputs (yield) were economically valued and used to determine farm level budgets.
- Budgets arising from rice grown with BioGro versus rice grown under conventional farmer practice (using chemical fertiliser) were statistically analysed using economic models

Models of commercial BioGro production

- To assess the factors that influence successful commercial product, two theoretical frameworks for examining BioGro production were used: supply chain management and transaction costs theory

- Factors considered included product characteristics (perishability, product differentiation, variability and visibility, novel characteristics); regulatory drivers (liability and traceability) and technology drivers (company-specific technology)
- A number of empirical production models were analysed under these theoretical frameworks. The models included two commune based production models and three private company production models.
- Outstanding issues within the production models were categorised and ranked in terms of the importance to the economic viability and sustainability of BioGro production.

5.1.4 Objective 4 : To conduct laboratory and field research to reveal the mechanisms of the PGPR biofertiliser effect

A number of hypotheses have been proposed to explain the mechanism/s by which biofertilser microorganisms act to promote plant growth. The true PGP effect may vary according to the microbial strain involved, the plant species or cultivar involved and the environmental conditions at the site of application. Consequently, the organisms in BioGro were examined for their potential to promote plant growth according via several scientifically accepted pathways

Nitrogen Fixation

- DNA was isolated from pure cultures of each BioGro strain and subject to PCR using primers designed to amplify DNA fragments from the nifH sequence reaquired for nitrogen fixation. Any PCR products resulting were sequenced and compared to ensure similarity to the nifH gene.
- The capacity of each organism to fix nitrogen under N-limited conditions was measured via acetylene reduction
- The potential for the strain *Pseudomonas fluorescens* 1N to enhance N-acquisition by rice was studied using N-15 enriched urea fertiliser in a glasshouse experiment

Phosphorus mobilisation

- Preliminary screening of biofertiliser strains for P-mobilisation was conducted by streaking of pure cultures onto agar plates containing tri-calcium phosphate precipitate. Putative P-mobilising organisms were identified by their ability to produce a clear zone in the medium by solubilising the phosphate precipitate
- The capacity for P-mobilising strains to solubilise P was investigated by culturing the organisms in liquid media containing insoluble TCP or $AlPO_4$ as the sole source of phosphorus. Soluble-P was measure as a function of time, pH and cell density
- The organic acids excreted by the P-mobilising strains were identified and quantified by HPLC

Phytohormone production

- Auxin production by each of the BioGro strains was quantified colorimetrically, with or without the supply of the precursor, L-tryptophan

Pathogen inhibition

- Selected BioGro strains were characterised for their ability to inhibit the growth of a number of different pathogenic fungi by plate co-culturing methods

Intensive characterisation of P. fluorescens 1N for PGP-potential

- *P. fluorescens* 1N was studied intensively for potential PGP mechanisms, including N-fixation, P-mobilisation, cyanide production, ACC-deaminase activity, aerotaxic ability, siderophore production, antifungal activity and root colonisation potential

5.2 Proposal Extension (Variation 4)

Objectives 1 and 2 were performed in Vietnam after provision of BioGro product to participating farmers according to the World Bank project DM08#5227 schedule. Approximately 200 farmers in the Mekong Delta region participated in workshops during the entire project WB project, but only a fraction of these (~30) conducted field experiments in conjunction with ACIAR objectives. These farmers were required to plant a control plot with regular practice N fertiliser rates, and a BioGro treated plot at half their usual N fertiliser rate. The farms were located in different provinces selected on the basis of location, farm history and consent, to provide data for objectives 1 and 2. A minimum of three seasons data (equivalent to one annual cycle) were collected. A number of additional experiments were also conducted in the south central coast region to provide extra data to address objective 2.

5.2.1 Objective 1: To correlate soil type and texture with N-use efficiency, with a view to optimising the urea-N level to be recommended with BioGro

- At each farmer sites, composite soil samples (3 sub-samples combined for each) at 0-10 cm depth and 40-50 cm depth were taken for routine characterisation, including available-N, available-P, cation exchange capacity (CEC), texture, pH, CEC, % organic carbon and exchangeable potassium
- All agronomic inputs throughout the growing season were recorded by farmers, including fertiliser inputs, pesticide applications and irrigations, as instructed by survey forms distributed at recruitment and training.
- Radiation and rainfall data were accessed from Vietnamese Bureau of Meteorology resources, and supplemented with farmer records.
- At harvest, straw and grain yield were measured, along with analyses of total straw and total grain nitrogen and phosphorus.
- A number of multivariate techniques, including multiple linear regression, canonical correspondence analysis, and partial least squares regression, were used to statistically identify the major factors contributing to BioGro performance and subsequently to provide best recommendations for fertiliser applications in conjunction with BioGro to achieve maximum yield benefits

5.2.2 Objective 2: To quantify the improvement in N- and P-use efficiency as a result of the application of BioGro.

- Data compiled from the field trials outlined above was used to calculate N and P budgets for individual farms, according to farmer practice (full chemical fertiliser) or BioGro practice (reduced chemical fertiliser)

- Analysis of variance was used to determine statistical differences in N and P use efficiency between the two fertiliser regimes
- Additional pot experiments were performed to identify potential mechanisms of enhanced N-use efficiency under BioGro treatment. Three different soil types were taken from fields of varying performance and used to grow rice and reference plants, with or without biofertiliser at different rates of N15-enriched urea. Isotopic analysis of plant and soil material was conducted in order to enable accurate quantification of nitrogen acquisition pathways by rice plants with or without BioGro treatment.

5.2.3 Objective 3: To optimise the rate of application of BioGro with a view to further reducing farmers' input costs

- A number of potential carrier materials were identified as low cost or waste organic by-products from primary industries, including sugarcane waste, aquaculture mud, coconut husk, rice straw and other by-products.
- All potential carriers were analysed for available N, available P, organic matter content, pH and total microbial activity.
- Each carrier was inoculated with BioGro strains and their survival and activity will be monitored, using methodology produced as an outcome of the original project proposal
- Potted rice seedlings were grown under glasshouse conditions in non-sterile soil amended with BioGro, with different carrier types and rates comprising treatments. All active carriers contained the same number of viable biofertiliser microorganisms, whilst controls consisted of sterile carrier materials. Rhizosphere samples were enumerated over time for BioGro strains using the methods described above.

5.2.4 Objective 4: To optimise the resource management commercialisation of BioGro production and application

- This objective was to be realised through the engagement of external business commercialisation experts with a background of business in Vietnam.

6 Achievements against activities and outputs/milestones

6.1 Original Proposal

Objective 1: To conduct field trials in Vietnam and Australia designed to optimise, extend and promote biofertiliser technology on rice farms for profit increase, extending this technology to the south of Vietnam

no.	activity	outputs/ milestones	completion date	Comments
1.1 PC	Conduct field experiments in Vietnam designed to optimise inoculant biofertilisers for profit increase on rice farms (HUS, IAS)	Comprehensive yield data from 22 field trials in Vietnam	June 30, 2007	All experiments completed on schedule; analysis of the data is reported in ACIAR Proceedings 130 and journal publications
1.2 A	Conduct field trials in Australia (US, Rice CRC)	Yield data from 2 field	June 30, 2006	Drought prevented completion of anticipated third experiment in Jerilderie; analysis of the data is reported in ACIAR Proceedings 130

PC = partner country, A = Australia

Objective 2: To design and evaluate a set of simple field tests for quality control of biofertiliser products aimed at ensuring their effectiveness under typical paddy rice field conditions,

no.	activity	outputs/ milestones	completion date	Comments
2.1 A, PC	Develop and field-test a set of simple tests for quality control of biofertiliser products (US, HUS)	Test kits/protocols for QC of starter cultures	June 30, 2007	Effective protocols were developed and optimised for fast and cheap laboratory screening, as summarised in the Quality Contol Manual (In Press).
2.2 A		Test kits/protocols for QC in factories (e.g. PGPR effects, identity, counting	October, 2007	Effective protocols were developed and optimised for fast and cheap laboratory screening, as summarised in the Quality Control Manual (In Press). As the result of an AusAID ALAF grant of $43,000, Ms Nga and Ms Cuc who worked in the project in Vietnam were able to extend their expertise in this area at a University of Sydney Workshop in April, 2009.
2.3 PC		Test kits for use by farmers (PGPR effect) and protocols for field tests	October, 2007	Field testing kits are currently being developed as an output of Variation 4 (see below)

PC = partner country, A = Australia

Objective 3: To investigate the economic and commercial feasibility of inoculant biofertiliser production in Vietnam,

no.	activity	outputs/ milestones	completion date	Comments
3.1 A, PC	Investigate the economic and commercial feasibility of BioGro in Vietnam (US, CU) and of extension to other countries	Benefit-cost analyses for economic benefits of biofertiliser use	October, 2007	Presented at project Review in October 2007 and included in ACIAR Proceedings 130.
3.2 A		Key indicators for successful commercial production	October, 2007	See Proceedings 130
3.3 PC		Policy involvement of MARD in Vietnam	Not achieved in project timescale. But local extension services have been found to be of far more relevance.	Success in World Bank competition may excite interest at policy level

PC = partner country, A = Australia

Objective 4: To conduct laboratory and field research to reveal the mechanisms of the PGPR biofertiliser effect.

no.	activity	outputs/ milestones	completion date	Comments
4.1 A	Ongoing laboratory and field research	None set	Ongoing	It was judged that significant use of project funds for this objective would be counterproductive. However, inferences to mechanisms can be made from the research and will be included in discussion of research papers

PC = partner country, A = Australia

6.2 Variation 4

Objective 1: To correlate soil type, texture and climatic factors with yield for better farmer recommendations

no.	activity	outputs/ milestones	completion date	Comments
1.1	Conduct first season field experiments to compare BioGro effect at 20 sites in two different provinces	Preparation of logbooks, sampling protocols and farmer training material (USyd, MDI)	September 2009	

		Initial soil samples collected and analysed, with all data compiled and documented (MDI, IAS)	September 2009	
		Minimum of four PAM measurements on 10 sites taken throughout growing period, data compiled and documented (MDI)	July 2010	
		Collection and compilation of yield and climate data (MDI, IAS)	August 2009	
		All first season data statistically analysed, interpreted, with report delivered to ACIAR and farmer stakeholder field day. (USyd, IAS, MDI)	April 2010	Field day at Cai Lay
1.2	Conduct second season field experiments, repeated at same 20 sites plus an additional 20 sites	All outputs/milestones as above but staggered 4 months later. Final data integrated together, analysed and reported (USyd, IAS, MDI)	September 2010-February 2011	Results to be published

PC = partner country, A = Australia

Objective 2: To quantify the improvement in N- and P-use efficiency as a result of the application of BioGro.

no.	activity	outputs/ milestones	completion date	Comments
2.1	Conduct field experiments to compare BioGro effect at 40 sites (as above) with different soil and climatic conditions	Analysis of plant and grain samples completed and compiled	February 2011	Results to be published
		Plant analysis data integrated with data from Objective 1 and analysed and reported with respect to nutrient use efficiency	March 2011	Positive results to be published

2.2	Glasshouse experiment using ∆15N method to accurately identify N acquisition by rice treated with BioGro	Experiment established using 3 different soils identified in Objective 1 as having a different yield and N nutrition response	Under mass spectrometric analysis in Sydney, July 2011	Delta N-15 measurements
		Plant-soil ∆15N data analysed and reported with respect to nutrient use efficiency	Not possible	Emission spectrometer in Ho Chi Minh City inoperative

PC = partner country, A = Australia

Objective 3: To optimise the rate of application of BioGro by estimating the numbers of biofertiliser organisms in inoculants and in the rhizosphere.

no.	activity	outputs/ milestones	completion date	Comments
3.1	Measure survival and activity of BioGro strains in new carrier materials	Identify and characterise potential new carrier materials (IAS, MDI)	September 2009	
		Inoculate best selected carrier materials with BioGro strains	December 2009	
		Use enumeration methods to quantify BioGro survival and activity in alternative carrier materials	January 2010	
3.2	Determine the efficacy of new BioGro carrier at different rates in glasshouse trials	Establish rice seedlings in glasshouse with inoculated and non-inoculate new carrier BioGro at different rates		Field trials preferred.; trials carried out by IAS and MDI
		Use enumeration methods to quantify BioGro survival and activity in rhizospheres	October 2010	Field trials carried out by Ganisan Krishnen at Jerilderie (see thesis), verifying that immunoblotting (see Deaker et al., 2011) allows enumeration
		Quantify plant growth performance, by dry weight, root length and shoot height. Correlate with BioGro inoculation rate		Field trials preferred; trial carried out by IAS and MDI

PC = partner country, A = Australia

Objective 4: To optimise resource management commercialisation of BioGro production and application using economic analysis

no.	activity	outputs/ milestones	completion date	Comments
1.1	Optimisation of resource management	Farmer surveys distributed and farmers trained in how to fill them out	April 2010	
		Producer factory surveys distributed and producers trained in filling them out	Partially achieved	
		Farmer and producer surveys, returned and input-output analysis conducted	Pending	
		Economic feasibility examined by independent evaluation of the franchising model	February 2011	Consultant Nigel Smith
		Sensitivity and uncertainty analysis completed and reported to stakeholders in field days etc	February 2011	

PC = partner country, A = Australia

7 Key results and discussion

Over the 5-year life of this project, considerable advances been made in promoting the use of biofertilisers to improve the nutrient-use efficiency of rice, thereby improving the economic and environmental livelihood of Vietnamese stakeholders. Highlights include the completion of over one hundred individual field trials, the majority involving local farmers, in order to optimise and promote the field application of biofertiliser technology; the development of new, rapid protocols and guidelines for ensuring the quality control of biofertilisers as key to technology uptake; the transfer of commercial biofertiliser production to southern Vietnam, thereby allowing access to greater numbers of rural poor farmers; and a number of scientific discoveries which extend the understanding and potential use of biofertilisers around the worldwide.

7.1.1 Optimisation, extension and promotion of biofertiliser technology on rice farms for increased profit

The use of targeted field trials to answer address critical knowledge gaps regarding biofertiliser application provided the perfect means to simultaneously **optimise**, **extend** and **promote** biofertiliser technology within Vietnam. Key findings for **optimisation** of BioGro application included:

- That BioGro application can reduce N-fertiliser application by between 25-50% without reducing yields. The magnitude of the fertiliser reduction possible without experiencing a yield reduction is highly dependent on the growing season: greater N-fertiliser reductions can (and should) be made in the dry season, because of higher solar insolation and improved soil quality resulting from flooding in the wet season immediately prior.
- That BioGro application does not significantly reduce the need for P- or K-fertiliser. Farmers should continue to apply an appropriate amount of these fertilisers that minimise economic loss or economic risk (note: this may or may not equate to maximising yield)
- That only one application of BioGro is necessary for effective plant growth promotion. A second application may enhance the PGP effect slightly, but more than two applications of BioGro are unnecessary and result in economic loss through increased biofertiliser costs without an effect on yield.
- That the effect of BioGro application will not carry over into the following season without re-inoculation. Re-inoculation is necessary from season to season to re-establish the PGP organisms in the rice rhizosphere.
- That BioGro of sufficient quality, which contains > 10^7 cfu g^{-1} of organisms, does not need to be applied at a rate of more than 100 kg ha^{-1} for maximum effectiveness. To do so only increases labour requirements without providing additional agronomic benefits. Over the life of the project, the optimum application rate was a recurring issue for discussion. Farmers preferred the biofertiliser product to be of a friable nature (i.e. a flowable product, but not too wet or dusty) and resented having to apply greater than 200 kg ha^{-1}. However, farmers also preferred to apply more than 20 kg ha^{-1} in order to achieve an even application over the entire field and as a matter of confidence (effectively as 'insurance' against applying too little to notice an effect). This matter also relates to production capabilities: recent advances in the quality of BioGro starter culture, from 10^8 cfu g^-

^{1} to 10^{9} cfu g^{-1}, by using sterilised peat, will allow manufacturers of final BioGro product to design a product favoured by the market. That is, if demanded by the market, manufacturers can reduce the quantity of carrier material by an order of magnitude, equivalent to the increase in quality of the starter culture.

- That the PGP response resulting from BioGro application is consistent irrespective of the rice cultivar being grown. That is, optimisation of the agronomic and environmental conditions during the growth season is more important than using one particular cultivar over another.

Aside from the optimisation of biofertiliser application with respect to rice agronomy, the field trials also provided an excellent opportunity to **extend** and **promote** biofertiliser technology amongst rice growers:

- In the original project (Variation 1), over 250 farmers, extension and technical staff in southern Vietnam participated in seven field days and workshops held in three different provinces of the Mekong Delta. This was in addition to over 50 farmers undertaking BioGro field trials in northern Vietnam, and workshops reaching more than 20 farmers in Tay Ninh province. With the completion of Variation 4 (in conjuction with the WBDM project) more than 100 additional farmers in the Mekong Delta, and 100 farmers on the south central coast have participated in BioGro workshops and field days. Over the life of the project, a total of over 500 individual farmers have received primary instruction on BioGro biofertiliser technology from the project investigator teams. The number of farmers indirectly reached through word of mouth has not been quantified; however, recent workshops in the Mekong Delta at Phung Hiep and Cai Lay have attracted participation from farmers through their own enquiry, rather than being approached by project staff.
- Compilation of all field results, including input and output data obtained from farmer surveys, provided a snapshot of the potential economic and environmental benefit afforded by the biofertiliser technology. This is of crucial importance as it is well know that private economic benefit is a key driver of technology promotion and uptake. During the initial phase of the project (2005-2007), the average net increase in income of farmers in the Mekong Delta using BioGro agronomy over conventional fertiliser agronomy ranged from 0.65-1.52 million VND ha^{-1} in the dry season to 2.29 million VND ha^{-1} in the wet season. This represented an increase in income of 5-50%, depending on the season and location. In northern Vietnam, increases in net income were in the range 0.36 – 0.92 million VND ha^{-1} when using BioGro agronomy, representing 19-33% increase in net income.
- A major highlight was the uptake and stewardship of the biofertiliser technology amongst the growers directly involved in the field trials. As a consequence of Variation 4, in conjunction with the WBDM project, improvements in biofertiliser effectiveness were observed as a direct outcome of *participatory farmer action*. Table 1 below shows the improvement of farmer yields and profitability throughout the WBDM/ACIAR V4 project as they gained greater experience and confidence in optimising the BioGro technology

Table 1 – The % of farmers achieving yield and profit increases after introduction to the technology in the S-A season 2009 through an annual cycle of optimisation. Number in brackets represent average Yield increases (T ha^{-1}) and Profit increases (million VND ha^{-1}) when using BioGro agronomy as compared to conventional agronomy

Site	Parameter	Crops			
		Summer-Autumn '09	Winter-Spring '10	Spring-Summer '10	Summer Autumn '10
Cai Lay	Yield	30 (0.1)	52 (0.4)	60 (0.3)	86 (0.3)
	Profit	30 (0.9)	60 (1.5)	60 (1.9)	79 (2.6)
P. Hiep	Yield	25 (0.1)	75 (0.5)	70 (0.4)	80 (0.2)
	Profit	25 (0.7)	80 (2.1)	70 (1.7)	80 (1.4)

In addition to the quantifiable benefits of BioGro extension and promotion, in terms of yield and profit increases, a number of other indirect benefits were realised, including:

- Farmer training on overall nutrient management, including the optimisation of chemical fertiliser application regardless of biofertiliser application. This included the realisation of many farmers that over-fertilisation was neither agronomically necessary or economically effective.
- An opportunity to promote 'VietGAP' amongst participating farmers as an means to access better market prices for quality rice. The goals of VietGAP are given in the Vietnamese slogan '1 phai, 5 giam', translated as '1 must, 5 reductions'. Participating farmers recognized that BioGro technology could contribute to reaching these goals by:
    - Reducing fertiliser – BioGro improves fertiliser use efficiency and reduces the requirements for urea fertiliser
    - Reducing pesticide – farmers consistently report more robust plants and less incidence of disease in BioGro- grown plants at lower N levels when compared to conventional plants
    - Reducing seeding – farmers reported better germination and reduced seedling death, reducing the need for over-seeding to compensate
    - Reducing water – the MDI (Dr Nhan) advises more aerobic growth of rice when BioGro is applied to encourage microbial growth.
    - Reducing harvest losses – farmers reported stronger, fuller grain and less shattering in grain produced using BioGro; growing rice with less flooding of paddies also promotes machine harvesting of rice improving grain yield by several percent.
- The improved farmer awareness of biological products in general resulted in farmers requesting specific biocontrol products for the treatment of pests and diseases such as rice blast.

7.1.2 Ensuring biofertiliser effectiveness through improved quality control

The benefits described above can only be achieved if adequate quality control of biofertiliser production and application is available. Quality control (QC), including the enumeration and identification of selected organisms in biofertilisers, is crucial to predict their effectiveness. Primarily, these methods are needed to validate the identity and quantity of specific plant growth promoting microorganisms in the inoculant, so that farmers can be certain the product is of sufficient quality and can test the product with confidence under their local environmental conditions. Secondly, counting methods are necessary for research purposes to optimise the efficacy of biofertiliser inoculants. Of particular importance is the survival of strains in the inoculant over time to determine shelf life, or enumeration of PGP cell numbers after application to follow their persistence in the field. Farmers can then use field trials as part of the QC effort, proving that the biofertiliser actually promotes the yield of the crop with reduced fertiliser inputs.

A major output from this project was the publication of a Quality Control Manual (currently In Press), which summarises international knowledge on biofertiliser regulation and provides clear guidelines and protocols for effective biofertiliser QC in Vietnam. All the methods presented in the QC were optimised and assessed as part of this ACIAR project and can easily be adapted for developing biofertiliser industries in other developing countries with respect to national regulations. Key results from this objective are summarised below.

Initial efforts to develop any QC guidelines should thoroughly review current national and international regulations regarding the definition and production of biofertilisers. Of particular importance is:

- The selection of microbial strains that are non-pathogenic. Potential strains from initial screening need to be carefully identified, preferably by molecular techniques to ensure that they pose no threat to human, animal or plant health. Identification has the additional benefit of providing additional information about the strain physiology, through a review of up to date literature.
- The definition of the standards required for product registration. Standards may incorporate a number of different parameters relating to what is known about product efficacy. In the case of biofertilisers, efficacy would generally be recognised as plant growth promotion under glasshouse conditions. Numerical standards have been identified as important for microbial inoculant quality, in that a minimum number of organisms (per g of product) must be present. Standards for product formulation should also be defined, and could include parameters such as pH, nutrient (N and P) content and moisture potential of carrier materials.
- The availability of low-cost, but robust, methods for the enumeration of biofertiliser strains. In accordance with this, guidelines must be defined on when and how often enumeration should be conducted. There are several points during the production of biofertilisers where tests should be done to determine quality. Tests on the original cultures or 'mother' cultures should ensure purity, high numbers and function of selected microorganisms before producing starter culture for distribution. Any problems at this point can be easily rectified. Checks throughout production on starter cultures and final products are also essential so that any problems in production can be quickly identified.

The points presented above are a distillation of the overall guidelines developed as a result of this ACIAR project. The development of these guidelines was made possible through numerous experiments using BioGro as a case study. Specific results regarding the quality control of BioGro include:

- The identification of two of the strains originally comprising BioGro were found to be potential pathogens of immuno-compromised patients. Despite the low-risk nature of these strains (*Citrobacter freundii* 3C and *Klebsiella pneumoniae* 4P), they were removed from the BioGro formulation. Identification of the new strains comprising the current BioGro formulation proved to be extremely useful, as similar isolates (*Pseudomonas fluorescens, Bacillus subtilis, Bacillus amyloliquefaciens*) have already been amply investigated elsewhere, showing PGP properties similar to the BioGro strains, thereby validating the selection procedures used by Professor Hien. The standards defined for BioGro are given below in Table 2

Table 2 – Recommended BioGro standards

BioGro Strain	CFU/g of carrier	kg per ha	Nominal CFU/ha
Pseudomonas fluorescens 1N	10^7	100	10^{12}
Candida tropicalis HY	5×10^6	100	5×10^{11}
Bacillus subtilis B9	10^7	100	10^{12}
Bacillus amyloliquefaciens E19	10^7	100	10^{12}

- A thorough evaluation of potential enumeration methods using the BioGro strains as a case study. The diversity of strains in BioGro provided an excellent opportunity to test the broad-scale applicability of these enumeration methods. The methods that were optimised and evaluated included:
    1. Conventional plate counting
    2. Selective plate counting on modified media (e.g. all strains were screened for antibiotic resistance, allowing the identification of useful antibiotics to select for only one strain)
    3. Differential plate counting on differential media (e.g. using Pikoskaia media to identify isolates capable of solubilising insoluble phosphorus)
    4. Most-probable number fermentation tubes, based on differential nutritional profiles
    5. Immunoblotting (conventional plate culture followed by blotting colonies onto a membrane and detection of biofertiliser strain blots by immuno-detection)
    6. Enzyme-linked immunosorbent assay (ELISA)
    7. Quantitative PCR based on specific 16SrDNA amplification
- It was decided that an ideal QC program would involve two screening programs: the initial screening would use rapid (less than 6 h turnaround), low cost methods for semi-quantitative counts, allowing more batches to be tested; the second method would involve longer culturing methods (1-3 d) to provide accurate counts of viable cells of batches failing the initial round of screening.

- Methods 6 and 7 were assessed for their potential to act a *rapid* methods for initial screening of product quality. QT PCR was highly specific and highly sensitive, however the cost and impracticality of using this advanced method excluded it from routine use. ELISAs were found to be sufficiently sensitive and specific, and of low cost. The conventional formatting of this method in a 96-well plate enables its use in a QC laboratory, but is not suitable for rapid quality checking on the factory floor. Current efforts are being made to format the ELISA test as dipstick technology.
- Of the methods assessed to measure living cells (methods 1-5), conventional plate counting and MPN fermentation were found to lack the specificity required to enumerate PGPR organisms in a potential background of contaminants. Immunoblotting was found to be specific and sensitive, however requires additional training and uses large volumes of immunoreagents, making it too expensive for routine counting. A combination of selective and differential media was concluded to be the best method for second-round screening, or as a sole screening method where ELISA testing is not available.

7.1.3 The economic and commercial feasibility of biofertiliser production in Vietnam

A number of potential production models were investigated in the initial phase of the project (2005-2007):

- *Commune-based production: Farmers produce and sell to local farmers*. All factories operating in this way were found to be unviable operating in this model. The major problem wa a lack of business acumen, resulting in difficulties with marketing in general and collecting monies owed by farmers in particular
- *Commune-based production: farmers produce only and sell to company.* One factory moved to this model, but still faced difficulties because of delayed payments from purchasers
- *Private company production: produces and sell to government contracts.* Only one factory operated according to this model, in Ha Tay province. On average the factory produced 280 t yr^{-1} from 2003-2006, but this was supported through personal contacts the producer had with government. The factory ceased production in 2007.
- *Private company production: produces and sells only BioGro to farmers:* A number of factories have operated to this model, with varying degrees of success. Of those that have ceased operation, all cited difficulties in marketing and management. Most of these companies failed to understand the need for conducting field trials and workshops to train farmers and promote the product through use. The common aspect of the successful companies was an enthusiasm and understanding of the BioGro product, with owners generally being younger and eager to learn.
- *Private company production: produces and sell BioGro and other products to farmers:* One factory operated under this model from 2003-2005, producing over 300 t y^{-1} at its peak. After initial success, the model ran into difficulty because, despite efforts from the BARC team, the company director advocated the use of BioGro in inappropriate ways (e.g. as a complete replacement for chemical fertiliser) in an effort to develop the market. Trials conducted by the producer were not adequately supervised and following these failures, farmers stopped buying the product and production ceased.

Additional supply chain factors were identified and addressed during this initial project phase, including:

- The protection of the intellectual property did not appear to be a problem, as the success of the technology relied heavily on building personal relationships between the BARC laboratory and the factory. Nevertheless, more effort to resolve this issue was deemed necessary in order to expand production to southern Vietnam
- Systems for QC were already put in place by BARC, and were improved by the project outputs, making it easier to accept by factory producers
- Supply and transport was not a problem in northern Vietnam. However the supply of BioGro from northern Vietnam to southern Vietnam was deemed too expensive and a limit to the expansion of the project. This necessitates the transfer of best production models to southern Vietnam.
- The product is well branded, but issues with product differentiation were identified during the project, with more effort needed in highlighting the differences in inoculant biofertilisers and organic fertilisers
- The opportunity to promote the use of biofertilisers in new ways and for new uses, such as horticulture and aquaculture, using different formulations. However, focus remained on biofertilisers for rice production, and this objective was made clear to all factories engaged during the life of the project.

These results led to the following recommendations:

- A commercial model of production based on private production was deemed necessary for southern Vietnam, rather than any farmer or commune based systems. The reasons for this decision included a need for sound economic returns on investments, in order to remove inefficiencies, speed up the rate of technology development and uptake, and limit wasted effort by project participants
- A franchise model was proposed as the favoured model for expanding BioGro production in the south. It was proposed that franchising would address some of the issues raised earlier, by regulating production in such a way to maintain high quality through QC, streamline marketing, reduce misuse by producers and provide an efficient mechanism to feed in technology developments. To achieve this, it was also decided that a parent company to oversee and administer the franchising and QC was necessary. The introduction of franchising as a potential mechanism to expand biofertiliser uptake was an integral part to the winning of the WBDM grant in 2008.

In 2009, following the extension of the ACIAR project as V4, in conjunction with the WBDM project, a program to attract potential franchisors BioGro producers commenced in the Mekong Delta. This involved meetings and site visits with potential franchisors to discuss project objectives, including production and distribution capacity, production sustainability and other issues arising. During this period, negotiations with more than 10 potential franchise companies were conducted. Key positive results included:

- Obvious interest in the potential for biofertiliser production as a financially viable activity, from both new and established entrepreneurs
- The potential for factories in the Mekong delta to distribute product easily and cheaply via the canal network, to both the Mekong and Saigon River catchments

- The potential for factories in the Mekong to incorporate waste products (such as sugarcane filter cake, aquaculture mud, coconut coir dust, rice straw) as a carrier material. This would reduce production costs and also contribute environmental benefits through waste recycling

Issues raised included:

- Recurring concern over intellectual property of the BioGro product strains
- A need for clear criteria under which franchisors could agree/disagree or negotiate about product specifications

Further progress was made by engaging an independent consultant to evaluate the commercialisation progress and feasibility. The principal conclusion are summarised below:

- The scientific basis for the positive impacts of use of Biogro in rice production in Vietnam appear to be sufficiently sound to justify current efforts to achieve large scale commercialisation
- The product is likely to gain the quickest acceptance if it can be marketed as delivering higher yields with lower inputs/cost, which appears feasible using a simple standard recommendation of a 25% reduction in nitrogen fertilizer application.
- Key factors affecting farmers uptakes are expected to include:
    - Product design: Easy to understand, easy to use, appropriate packaging
    - Production: Consistent performance, suitable pricing
    - Distribution: Available at the right time and place
    - Marketing: Word of mouth / trust , seeing neighbours use it
- From both a manufacturer's and farmer's perspective, there appear to be some unanswered question in terms of product "shelf life", storage and usability that will need to be resolved as a priority to secure the interest of partner companies to invest in the manufacture, distribution and marketing of the product.
- A technology license agreement appears to be the most suitable arrangement to explore for large scale commercialization in which:
    - The parent company :
        - transfers technology to the select licensees, including production and quality control techniques as well as parent strains
        - carries out quality control checks on products to ensure proper use of the technology, with any non-compliance with the terms of license being addressed and, potentially, ultimately resulting in the withdrawal of the license
        - markets the brand to farmers as a quality assurance brand for reliable, high quality biofertilizer – "look for the badge"
        - provides ongoing technical backstopping to licensees if they encounter problems in manufacturing or in feedback from the farmers
        - continues to do develop the product and technologies, and transfers to licensees (subject to terms of the license agreement)
    - The Licensees:
        - Manufacture and distribute the product

    - Lead the marketing of the product under their own brand/packaging, but supported by complementary marketing by Green Future
    - Determine sales price for farmers
    - Work with Green Future to ensure acceptable quality control of product
    - Work with Green Future on new product development, such as new formulations or use of alternative carrier materials to respond to market demand

- There is already a relatively competitive market for seed and fertilizer supply in the Mekong region. This creates the opportunity to try and identify suitable businesses that may be interested in taking on a license for the BioGro technology that have the capacity, resources, reputation and distribution networks to lead the commercialization of BioGro into the mass market. The ideal characteristics of a licensee business may include:
    - Good brand/reputation among farmers
    - Distribution network, especially reaching early adopters
    - Mekong production facilities able to produce good quality standardized product
    - Understanding of technology and benefits
    - Large market share
    - Good financial strength
    - Happy with QA aspect of license

7.1.4 The mechanism of action of PGPRs

A number of different mechanisms of action of PGPRs have been proposed in the literature. The final objective of this ACIAR project was to identify the mechanisms present BioGro-rice interaction, in order to better predict its field performance. Laboratory, glasshouse and field methodology was used to achieve this. Major laboratory characterisation results are given in Table 3 below.

Table 3 – PGP characteristics of the BioGro strains

Proposed Mechanism	Research Method	Result			
		P. fluorescens 1N	*C. tropicalis* HY	*B. subtilis* B9	B. *amyloliquefaciens* E19
N-fixation	*NifH* gene amplification	No	No	No	No
	Acetylene reduction	No	No	No	No
P-mobilisation	Clear zone on Pikiskaia media	Yes	Yes	No	No
	Organic acid production	n.d.	High levels of fumarate	n.d.	n.d.
	Phytase production	No	Yes	No	No
Phytohormone	Colorimetric	Yes	Yes	No	No

production (IAA)					
ACC deaminase production	Growth on N-free media with ACC	Yes	n.d.	n.d.	n.d.
Siderophore production	Chelation of Fe(II)	Yes	n.d.	n.d.	n.d.
Pathogen Inhibition	Cyanide production	Yes	n.d.	n.d.	n.d.
	Co-culturing	Inhibits *Colletotrichum capsicii*	n.d.	Inhibits *Fusarium oxysporum*	n.d.

Further work was conducted focussing on *P. fluorescens* as a model PGPR strain. Additional characteristics included:

- High motility, with an aerotactic (i.e. moving towards oxygen) response. This indicates its capacity to move towards rice roots, which provide oxygen in the rhizosphere through aerenchyma cells
- Good colonization ability of rhizosphere
- An ability to increase access to N in the rhizosphere, as demonstrated by N-15 studies. At 20 days after sowing, inoculation halved the fertiliser-N uptake compared to non-inoculated plants, but there was no difference in total N uptake, meaning that additional sources of N were accessed. After 45 days of growth, BioGro inoculated plants had taken up nearly 20% more N that non-inoculated plants; but by this stage the amount of fertiliser-N taken up was the same in both treatments. The fact that laboratory studies could not confirm N-fixation by *Pseudomonas fluorescens* implies the following possibilities:
    - *P. fluorescens* may indirectly stimulate other soil microorganisms to increase N-fixation in the rhizosphere
    - *P. fluorescens* may directly mineralise organic N, or indirectly stimulate other soil microorganisms to mineralise organic N
- The role of BioGro in improving N nutrition has been confirmed in numerous field trials over the 5-year project. An example of the BioGro × N response obtained in the Mekong Delta in 2010 field trials is shown below in Figure 1.

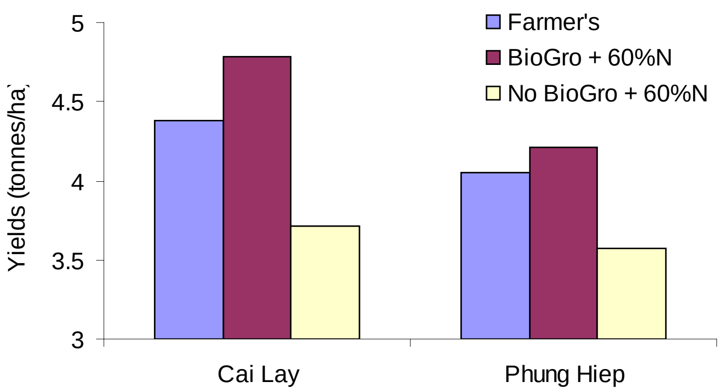

Figure 1 – The yield response of rice treated with Farmer's usual N-fertiliser recommendation (100%), versus BioGro with reduced N, and reduced N alone.

8 Impacts

8.1 Scientific impacts – now and in 5 years

- The biggest scientific impact will likely be an increased intensity of international scientific research and development for PGPRs as an agronomic tool to increase fertiliser use efficiency, in particular N. This will be particularly evident in new research originating from less developed countries, as PGPR technology moves from the laboratory and glasshouse into the field. Such an outcome will result from extensive communication activities of this project, including oral presentations at the International Rice Research Congress in Hanoi 2010 by Dr Phan Thi Cong, at a COST Workshop in Berlin in June, 2011 and in the Rhizosphere 3 Conference in Perth in 2011.
- The key publication of Phan et al. (2009), which proves the interaction of non-symbiotic PGPRs with N nutrition of rice, has already been cited 7 times, by Malaysian, Chinese, Korean, Brazilian, Turkish and Indian authors. These authors re-iterate our findings that effective PGPR organisms improve the N nutrition of crops, and that the exact mechanism is still ill-defined. We believe that our forthcoming publication (Dang et al, In Preparation) will further stimulate scientific research into the optimisation of biofertiliser effectiveness under field conditions, as it will include the results of over 100 field trials conducted over 4 growing seasons, a feat that has only been achieved (to our knowledge) with *Azospirillum* (Okon and Labandera-Gonzalez, 1994)
- We predict that further scientific impacts will be evident in the next five years in the area of quality control of biofertiliser products, arising from improved methodology presented in publications arising from this project (Keckskes et al., 2009; Rose et al., 2011; Deaker et al., 2011; Krishnen et al., 2011).

8.2 Capacity impacts – now and in 5 years

- One of the most significant capacity impacts realised in this project has been the extensive farmer training throughout the Mekong Delta and South Central Coast areas. Through this project, farmers have now have a much better awareness and appreciation for farm nutrient management in terms of agronomic, economic and environmental gains through more efficient nutrient use. Furthermore, the introduction of biological farming practices in this project has been a revelation for many farmers, who now recognise the importance of soil biology in agro-ecosystems and the potential to use other biotechnologies such as biocontrol agents. We believe that the increased contact between the IAS, MDI and regional farmers that was facilitated by this project will increase the capacity for rapid adoption of any best management practices and technology transfer that may occur in the future. The network generated by this project between government officials, scientists, commercial enterprises and farmers cannot be understated.
- The long-term investment of ACIAR in this project has allowed the continual involvement and interaction of numerous scientists and students for over 5 years. The strength of relationships developed in this project has proved, and will continue, to be one of the most critical aspects in enabling capacity building now and in the future with respect to scientific capacity in Vietnam and other developing countries. Highlights include the placement of Michael Rose as a AusAID Youth Ambassador in Vietnam for 10 months, the training of Vu Thuy Nga (IAE, Hanoi)

and Tran Thi Kim Cuc (IAS, HCMC) for 2 months in Sydney through an AusAID grant; the visit of USyd International Postgraduate student working on the BioGro PGPR organisms studying rice root proteomics (from Thailand), Khanok-on Amprayn, to BARC in Hanoi; the involvement of International Postgraduate student Ganisan Krishnen (from Malaysia) in biofertiliser research; and the involvement of senior research scientist Dr Gamini Seneviratne (Sri Lanka) in the ACIAR review and interaction with Ms Nga and Ms Cuc at the University of Sydney. Recently, AusAID has funded a 3-year Public Sector Linkage Project ($156,000) entitled *Effective use of microbial bio-fertilisers for an improved economy and environment in Sri Lanka* (2011-2014) consolidating this network of activity regarding biofertilisers in the south Asian region. This ACIAR project has therefore effectively facilitated a strong scientific network in SE Asia for biofertiliser technology, already extended three years into the future.

As a result, capacity impacts have already been realised, particularly at the IAS in HCMC, where Ms Tran Minh Hien and Ms Tran Thi Kim Cuc, under the leadership of Phan Thi Cong, have already applied skills learnt in the project for the isolation and characterisation of microbial isolates for other purposes, including biocontrol agents for pepper horticulture, and isolates for rapid composting of organic wastes. This is in combination with two large autoclaves necessary for this work being purchased with funds from the ACIAR project.

- We conclude that capacity now exists in Vietnam for the continued development and commercial success for biofertiliser technology; as a result of the establishment of a new company Agriculture Green Future, this capacity is more self-sustaining, and moreover can act as a catalyst for improving farmer livelihoods in the region. For example, the knowledge base and skills capacity developed in this project has recently triggered an invitation from the World Bank to examine the possible extension of biofertiliser technology to rice farmers in Cambodia.

8.3 Community impacts – now and in 5 years

Community impacts from the application of biofertiliser technology as BioGro are still limited, particularly as applied to rice production. Overall, the total number of Vietnamese at present who have direct knowledge of either the production or the application of BioGro to rice would be several thousand Vietnamese farmers. This current impact was initiated in the north, largely through the efforts of Professor Hien, the developer of BioGro, but has been significantly extended at least doubling in the 2008-2011 ACIAR/World Bank extensions. Thus, there is current community impact at about 20 centres in Vietnam in the north, central regions and now throughout Vietnam including the Mekong Delta. Because BioGro production licensed either by BARC or Agriculture Green Future will probably be produced locally, there is anticipated to be a community impact in the future as a result of associated cash flows with improvement in economic status of local communities.

8.3.1 Economic impacts

The economic impacts of the use of BioGro were illustrated by the positive data regarding benefits to farmers first obtained during the extension program in the Mekong Delta in Variation 23 of this project. These data are documented in ACIAR Proceedings 130 indicating substantial increased profits from application of BioGro instead of chemical fertilisers. This economic impact is increased in times of high fertiliser prices as has been the case particularly since 2007. These economic impacts have been amply confirmed in the World Bank project DM#5227 *Sustaining nitrogen-efficient rice production* (Phan et al., 2011). The need to reduce risk resulting from a choice by rice farmers to apply BioGro has been recognised in a recommendation to limit the reduction in urea-N applied to, say, 60-75% of normal farmer practice.

However, how sustainable the licensing of BioGro production and application promoted by Agriculture Green Future will be remains to be seen. The establishment of Agriculture Green Future with a guarantee of continued base level funding until 2014 at least provides a sustainable trial period for further promotion of the inoculant fertiliser technology.

8.3.2 Social impacts

Application of the biofertiliser technology is not considered to have significant social impacts, other than as a result of its impact on improving wealth.

8.3.3 Environmental impacts

It is anticipated that reduced rates of application of urea and improved efficiency in its use will lessen the probability of nitrous oxide emissions. The more BioGro is applied, the greater environmental benefits that will be achieved. This is a result of the reduced need for fossil fuels used in fertiliser production, thus lessening carbon dioxide production in direct proportion to the reduced need for urea. Overall, biofertiliser technology is anticipated to provide a more environmentally sustainable use of natural resources.

8.4 Communication and dissemination activities

Award/Recognition Name	**Type of award** (e.g., local, national, international)	**Date of award**	**Description of award**	**Web links/news clips, if available**
Cup Vang, San Pham An Toan Va An Sinh Xa Hoi	National Ministry of Agriculture Rural	Oct 2008	Innovative technology	

	Development (MARD)			
Golden Cup	National (MARD)	October 2008	Safety Product for Community Welfare	
Selection of Phan Thi Cong to speak at International Rice Congress, Hanoi	International	Nov 2010	Sole Vietnamese female speaker at Rice Congress	www.ricecongress.com/extPdfs/OP02-4213%20Phan%20Thi-edited.pdf
Binh Thuan, An Giang and Phu Yen, Extension Services	Vietnam	2009-2010		
ABC National Radio	National	June 2009	News coverage of project work	www.abc.net.au/rural/content/2008/s2532337.htm

As shown in the publication list, a continuous process of communication has accompanied this project. In addition, recognition of the project activity has followed various awards, shown in the table following. This dissemination has led to several new grants, from the World Bank and AusAID.

9 Conclusions and recommendations

9.1 Conclusions

Field experiments with the inoculant biofertiliser BioGro in Vietnam have confirmed its efficacy, optimising and promoting this technology on rice farms for increased profit. In subsequent action research with rice farmers, the agronomic response of rice to the microbes in BioGro has been well defined. This participatory approach has increased the likelihood of yield increases, based on data obtained from four successive rice seasons.

Thus, a farming system using BioGro to initiate technology transfer, designed by the Mekong Delta Research and Development Institute, provides up to five reductions (seed, fertilisers, pesticides, water and harvest losses). This is essential for achieving VietGAP (good agricultural practice) rice production in Vietnam. Furthermore, field results show that improvements in rice production on acid sulphate soils are possible with reduced N-inputs and BioGro.

To augment this field success, a set of simple field tests for quality control of biofertiliser products aimed at ensuring their effectiveness under typical paddy rice field conditions has now been achieved (Deaker et al., 2011).

Based on preliminary research in this project considering different models for economic and commercial inoculant biofertiliser production in Vietnam (Kennedy et al., 2008) a sustainable means of promoting this objective has been designed. Agriculture Green Future (AGF), a technology transfer company similar to BARC in Hanoi has been registered in Ho Chi Minh City. AGF will license the technology to selected manufacturers, providing starter cultures, quality control and market promotion.

Finally, laboratory and field research conducted by research students in Australia has helped to reveal the mechanisms of the PGPR biofertiliser effect.

9.2 Recommendations

Continued support of this emerging agricultural biotechnology is essential. Despite its demonstrated benefits, uptake by farmers may continue to lag without additional promotion. Yet it is increasingly obvious that this technology will become the accepted norm for soil and crop health in the future.

Support at a higher strategic and social benefits level than normal commercial activity is also desirable. The reasons this would be justified include:

<u>Greenhouse and climate change credits</u>

- Reduced fertiliser inputs
- Reduced nitrous oxide

- Increased biomass

<u>Promotion of a BioGro-based farming system by authorities through VietGAP is also justified by the results of these studies, showing the following advantages</u>

- Reduced seed
- Reduced fertiliser
- Reduced pesticides
- Reduced water
- Reduced harvest losses.

We also recommend the funding and promotion of a formal action network. The SUNFix Centre now has links with biofertiliser research and promotion in Vietnam, Malaysia, Thailand, Pakistan, India, Bangladesh, Indonesia and now Sri Lanka. In all these interactions the main emphasis is on quality control and the means to achieve this.

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10.2 List of publications produced by project

Books

1. *Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers.* Kennedy, I.R., Choudhury, A.T.M.A., Kecskes, M.L. and Rose, M.T. (eds.) (2008). Proceedings of a project (SMCN/2002/073) workshop held in Hanoi Vietnam, 12-13 October 2007, ACIAR Proceedings No. 130 137 pp., Canberra ACT. http://aciar.gov.au/publication/PR130

2. *Practical methods for the quality control oif inoculant biofertilisers.* Deaker, R., Kecskes, M.L., Rose, M.T., Amprayn, K., Krishnen, G., Tran, C.K.T., Vu, N.T., Phan, C.T., Nguyen, H.T. and Kennedy, I.R. (2011). ACIAR Monograph No. 147, ISBN978 921738 83 8 (print) ISBN978 921738 84 5 (online), 101 pp., Canberra ACT. http://aciar.gov.au/publication/mn147

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3. Phan Thi Cong and Tran Dang Dung (2008) *Interaction effects of BioGro with N and P on grain yield and nutrient uptake of rice in light textured soils of southern Vietnam*. Kennedy, I.R., Choudhury, A.T.M.A., Kecskes, M.L. and Rose, M.T. (eds.) (2008) Proceedings of a project (SMCN/2002/073) workshop held in Hanoi Vietnam, 24-31 October 2007, ACIAR Proceedings No. 130, pp. 9-14, Canberra ACT.
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3. Ganisan Krishnen. *Quorum Sensing of Biofertiliser Organisms by Plants.* Supervisors: I.R. Kennedy, Mihály Kecskés and Lily Pereg.

4. Khanokon Amprayn. *Root Proteomics of Rice with* Pseudomonas fluorescens *1N and* Candida tropicalis. Supervisors: I.R. Kennedy, Mihály Kecskés and Lily Pereg.

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March 2009 Newsletter: Article on World Bank project 5227: *Sustaining nitrogen-efficient rice production.*

ACIAR Site

Helping farmers help themselves. *http://aciar.gov.au/node/10636*

ABC Site

See also ABC site. *http://www.abc.net.au/rural/content/2008/s2532337.htm*

11 Appendixes

11.1 Appendix 1:

Practical methods for the quality control of inoculant biofertilisers. Deaker, R., Kecskes, M.L., Rose, M.T., Amprayn, K., Krishnen, G., Tran, C.K.T., Vu, N.T., Phan, C.T., Nguyen, H.T. and Kennedy, I.R. (2011). ACIAR Monograph No. 148, ISBN978 921738 83 8 (print) ISBN978 921738 84 5 (online), 120 pp., Canberra ACT. (http://aciar.gov.au/publication/mn147)