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| <b>Project Title<br/>Code: 1.15</b> | <b>Strengthening Institutional Capacity in the Production of Improved Forest Tree Germplasm in Vietnam through Application of Molecular Genetics Technology</b> |
| <b>Australian Personnel</b>         | Dr Chris Harwood, Project Leader; Dr Penny Butcher  |
| <b>Australian Institution</b>       | CSIRO Forestry and Forest Products  |
| <b>Vietnam Institution</b>          | Research Centre for Forest Tree Improvement (RCFTI)-<br>Vietnam Institute of Forest Sciences  |
| <b>Project Duration</b>             | September 2000 - August 2002  |

## Executive Summary

Activities completed in the reporting period have included

- seed collections from six *Acacia mangium* seed production areas (SPAs) in Vietnam
- germination and raising of over 2000 seedlings from the SPAs at RCFTI, Hanoi
- further training of four Vietnamese staff in molecular techniques in Hanoi
- extraction at RCFTI of DNA from 70 maternal parent trees and 700 progeny from the SPAs
- design of field trials for planting of seedlings to allow assessment of inbreeding depression
- arrangements made for training of RCFTI staff in Canberra in October 2001.

Dr Chris Harwood visited Vietnam in May 2001 to work with RCFTI scientists to complete the collection of leaf and seed from the six SPAs and supervise procedures for extraction and labelling of seeds and germination of seedlings. Dr Penny Butcher visited Vietnam in September 2001 to continue training of four scientists from RCFTI in molecular techniques, supervise and assist with DNA extractions. The DNA extractions from 70 maternal trees and 700 progeny were completed using the Fast Prep machine and quantified using the fluorometer purchased in the first phase of the project. The new laboratory built at RCFTI in Hanoi, and the equipment installed through the project, were adequate for the molecular work. Additional equipment (sequencing gel apparatus) is being purchased for DNA fingerprinting to allow the techniques acquired under the CARD project to be used for routine monitoring of RCFTI breeding programs.

## **Project Description**

The Overall Goal of the project is to increase the sustainable productivity of forest plantations and farm forests in Vietnam through strengthening the capabilities of Vietnamese forest scientists to produce genetically superior seed and planting material of priority plantation species. The Development Objectives are to

1. Strengthen the research capability of the Research Centre for Forest Tree Improvement (RCFTI) in the field of molecular genetics through mutual visits of scientists, and by cooperative field and laboratory work in both Vietnam and Australia.
2. Use appropriate molecular genetic techniques to examine the quality of seed production areas in Vietnam and to fingerprint selected breeding clones. This will be used to develop protocols for the production of genetically superior seeds and planting materials.

The project involves: (i) training of one molecular geneticist and one technician from RCFTI in CSIRO laboratories for four weeks in the appropriate molecular genetic techniques; (ii) four scientists and technical assistants from the RCFTI will be trained in Vietnam in the use of molecular techniques to monitor breeding practices and programs to ensure maximum genetic gains; (iii) laboratory work will be carried out in Canberra and Vietnam to fingerprint existing clones of the acacia hybrid and to determine breeding systems of *Acacia mangium* and the levels of outcrossing in the seed produced by six established *A. mangium* SPAs and seed production areas across Vietnam; and (iv) two field trials in Vietnam will be established to determine the actual level of inbreeding depression in *A. mangium* by measuring the productivity and growth characteristics of self-fertilized and outcrossed plants.

## **Appropriateness of Project Design and Objectives**

### ***Project Background and context***

The Government of Vietnam has embarked on a massive tree plantation program. By 2010 it plans to establish an additional 5 million hectares of plantations on cleared land, over and above the current estate of one million hectares, plus the equivalent of more than 50,000 hectares of community forests in scattered plantings. The current planting rate is about 120,000 hectares per year, so if the targets are to be met there will have to be a dramatic increase in planting rates. This will require equally dramatic increases in the amounts of genetically-superior seed. The Government is committed to improving the quality and quantity of tree seed produced from its own seed orchards. For example, one hectare of well-managed *Acacia* seed orchard can produce 10-20 kg of seed per annum, which is sufficient to establish 200-400 hectares of plantations.

# PROJECT COMPLETION REPORT

## Executive Summary

The project has achieved all of its planned outputs and outcomes, which can be summarised as follows:

- Strengthen the research capability of RCFTI in the field of application of molecular markers for forest tree breeding
  - Achieved through purchase of appropriate project equipment, transfer of DNA microsatellite markers, and training of Vietnamese scientists and technicians in Vietnam and Australia
- Apply molecular genetic techniques for effective production of seeds and planting materials for forest plantations
  - All the acacia hybrid clones used operationally in Vietnam were fingerprinted to allow their unambiguous identification
  - Hybrid identity and paternity of promising new clones was checked, some identities were confirmed and others found to have different parentage to that predicted from controlled pollination, indicating pollen contamination during crossing or labelling errors
  - Outcrossing and selfing rates were assessed precisely in six *A. mangium* seed orchards across different locations in Vietnam. Most orchards had selfing rates of less than 5%, but one orchard which displayed poor flowering produced 87% selfed seed
  - Two field trials were planted to quantify the relative performance of outcrossed and selfed seedlings, identified through molecular genetic analysis
  - Guidelines for seed collection from *A. mangium* seed orchards in Vietnam, to improve the quality of seed collected, were developed
  - A training manual for microsatellite analysis was prepared.

The performance of the lead institutions CSIRO Forestry and Forest Products (CSIRO FFP) and the Research Centre for Forest Tree Improvement (RCFTI) has been satisfactory.

## 1. Project Description

### 1.1 Background and preparation

The background to the project is the long history of productive research collaboration in the area of forest genetic resources development and tree breeding, commencing in 1988, between CSIRO Forestry and Forest Products (CSIRO FFP) and the Research Centre for Forest Tree Improvement (RCFTI), Forest Science Industry of Vietnam. Discussions between FFP and RCFTI in 1999 identified the area of application of molecular genetic technology in tree breeding as being of strong mutual interest. RCFTI wished to build capacity in this area and apply it to the practical genetic improvement of priority

plantation species for Vietnam, especially tropical acacias. Technology and skills that CSIRO FFP already had developed for tropical acacias were thus highly relevant to RCFTI. CSIRO FFP saw that elements of the project would support strategic development of its molecular genetic research and provide results with international application. The project proposal was prepared jointly by the two organizations and submitted for funding in March 2000.

## **1.2 Context and rationale**

The project has operated in the context of the ongoing working relationship between the Australian and Vietnamese partner agencies, which are jointly implementing several other projects including one other project supported by CARD (*Future needs of the Forest Products Industry in Vietnam: Building Professional Capacity and Raising Awareness among Policy Makers and Research Scientists*) and two projects supported by ACIAR (*Domestication of Australian Trees Project* and *Development of Domestication Strategies for Commercially Important Species of Meliaceae*). The project under review was seen by both partners to complement these other projects and to form a basis for further collaborative research on advanced breeding of tropical acacias.

## **1.3 Project objectives and scope at design**

The Overall Goal of the project was to increase the sustainable productivity of forest plantations and farm forests in Vietnam through strengthening the research capabilities of forest scientists, the breeding and propagation of the acacia hybrid and the production of genetically-superior seed of *Acacia mangium*. The Overall Goal was to be achieved through the following Development Objectives:

1. To strengthen the research capability of the Research Centre for Forest Tree Improvement (RCFTI) through mutual visits of scientists, and by cooperative field and laboratory work in both Vietnam and Australia.
2. To apply appropriate molecular genetic techniques for effective production of genetically-superior seeds and planting materials for forest plantations.

The goal and the Development Objectives were consistent both with AusAID's country strategy for Vietnam, which places emphasis on rural development and poverty alleviation, and the strategy of the Government of Vietnam, which has embarked on a massive tree plantation program on bare land allocated to forest production. By 2010 it plans to establish an additional 5 million hectares of plantations on cleared land, over and above the current plantation estate of one million hectares. The current planting rate is about 120,000 hectares per year, so if the targets are to be met there will have to be a dramatic increase in planting rates. Seed-based plantation of the fast-growing tropical species *Acacia mangium* and clones of its interspecific hybrid with *A. auriculiformis* will make up a substantial proportion of these new plantations. With such large areas of plantations and community forests of *A. mangium* and its hybrid being established, involving very significant investments by national agencies and farmers, it is extremely important to make sure that the planting stock is of the highest possible genetic quality. The Government of Vietnam is committed to improving the amounts and qualities of tree seed produced from its own seed orchards. For example, one hectare of well-managed

*Acacia* seed orchard can produce 20 kg of seed per annum, which is sufficient to establish 400 hectares of plantations.

#### 1.4 Implementation arrangements

The project was jointly managed by joint project leaders Dr Chris Harwood (Principal Research Scientist, CSIRO FFP) and Dr Ha Huy Thinh (Director, RCFTI). The project leaders met at regular intervals during the project. Dr Harwood visited Vietnam in November 2000, May 2001, November 2001 and March 2002, and Dr Thinh visited Australia in August 2001 and April 2002. The leaders kept in regular touch through telephone and e-mail. CSIRO and RCFTI prepared and signed a contract for RCFTI to provide project services, with payment to RCFTI made in two tranches, the second conditional on satisfactory completion of the first round of RCFTI activities. Both payments to RCFTI were made as scheduled.

## 2. Appropriateness of Project Design and Objectives

### 2.1 Appropriateness of Objectives

| Objective No<br>(1, 2, 3, etc) | Objective description   | Appropriateness<br>Rating |
|--------------------------------|---|---------------------------|
| 1                              | To strengthen the research capability of the Research Centre for Forest Tree Improvement (RCFTI) through mutual visits of scientists, and by cooperative field and laboratory work in both Vietnam and Australia. | 5                         |
| 2                              | To apply appropriate molecular genetic techniques for effective production of genetically-superior seeds and planting materials for forest plantations.   | 5                         |

### 2.2 Appropriateness of Design

| Description of design feature (summary of detailed design features)   | Appropriateness<br>Rating |
|---|---------------------------|
| Selection and investigation of a research question for investigation which provided a suitable vehicle for training as well as giving results of immediate practical relevance to tree breeding and plantation improvement in Vietnam and other countries | 5                         |
| Technology (CSIRO's DNA microsatellite markers) and associated equipment transfer to Vietnam and their use to address the research question while building sustainable capacity in this science area  | 5                         |

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|---|---|
| Training of Vietnamese scientists and technicians in molecular genetic techniques, in Australia and Vietnam, and using these techniques to address research problem             | 5 |
| Training of Vietnamese scientists and technicians, in Vietnam, to carry out field sampling, to raise seedlings for DNA sampling and field trials, and to establish field trials | 5 |

5- Best Practice; 4- Fully Satisfactory; 3- Satisfactory overall; 2- Marginally Satisfactory;  
1- Weak

### 3. Implementation Performance

#### 3.1 Project Components and Outputs

| Component No. | Component Description  | Outputs   | Performance Indicators   | Performance Rating |
|---------------|--|---|--|--------------------|
| 1             | Project management   | Management of project components                          | Project components delivered as specified  | 4                  |
| 2             | Leaf and seed sampling from Vietnamese seed orchards   | Samples of trees and seeds from each of six seed orchards | Samples collected successfully   | 5                  |
| 3             | Raising of seedlings for DNA extraction and field trials   | seedlings labelled and raised                             | 720 Seedlings correctly labelled and raised successfully   | 3                  |
| 4             | Training in Vietnam on DNA extraction and quantification   | Training  | Equipment functioning, 4 Vietnamese scientists and technicians trained   | 5                  |
| 5             | DNA extraction from leaves of <i>A. mangium</i> parent trees and seedlings, and acacia hybrid clones                         | Extracted DNA   | DNA successfully extracted from over 750 leaf samples  | 5                  |
| 6             | Training in Australia on microsatellite analysis of DNA samples using automated DNA sequencer, and interpretation of results | Training and use of DNA sequencer for fragment analysis   | 3 Trainees able to operate automated DNA sequencer and interpret results<br>Training manual on components 4 & 6 prepared | 5                  |
| 7             | Fingerprinting of acacia hybrid clones   | Clone fingerprinting                                      | Clones successfully fingerprinted  | 5                  |
| 8             | Training in Vietnam on microsatellite analysis using sequencing gels, and interpretation of results                          |   | Vietnamese trainees competent to operate sequencing gel system and interpret results                                     | 4                  |
| 9             | Field trials to quantify effect of selfing on growth performance   | Two field trials  | Field trials successfully established  | 3                  |
| 10            | Scientific reporting   | Scientific papers   | Scientific papers completed  | 4                  |

### 3.2 Project Outcomes

The project has achieved all of its planned outcomes, which can be summarised as follows:

- Strengthen the research capability of RCFTI in the field of application of molecular markers for forest tree breeding
  - This outcome has been achieved through purchase of appropriate equipment, set up of a new laboratory at RCFTI, transfer of DNA microsatellite markers, and training of Vietnamese scientists and technicians in Vietnam and Australia.
- Apply molecular genetic techniques for effective production of seeds and planting materials for forest plantations
  - Six microsatellite markers for use on tropical acacias were successfully transferred for use in the RCFTI laboratory along with necessary equipment (fluorometer, 'Fast-Prep' DNA machine and extraction kits) and staff were trained in relevant techniques for DNA extraction, use of the microsatellite markers, and interpretation of results obtained
  - The eight elite acacia hybrid clones currently used operationally in Vietnam were fingerprinted to allow their unambiguous identification. Eleven *A. auriculiformis* and eight *A. mangium* clones used in hybrid breeding programs were also genotyped
  - Hybrid identity and paternity of promising new clones was checked, some identities were confirmed and others found to have different parentage to that predicted from controlled pollination, indicating errors during crossing
  - Outcrossing and selfing rates were assessed precisely in six *A. mangium* seed orchards across different locations in Vietnam. Four orchards had selfing rates of less than 5%, but one orchard which displayed poor flowering produced 87% selfed seed, and another orchard where late-flowering trees were sampled produced 51% selfed seed
  - Two field trials were planted to quantify the relative performance of outcrossed and selfed seedlings, identified through molecular genetic analysis. A follow-up replacement field trial will be planted in December 2002 to replace the trial at Dong Ha which was damaged by cattle grazing
  - Guidelines for seed collection from *A. mangium* seed orchards in Vietnam, to improve the quality of seed collected, were developed
  - A scientific paper reporting the results already obtained has been co-authored by Australian and Vietnamese collaborators, and presented at a IUFRO international conference on forest genetics held in Slovakia in August 2002. Two scientific papers are being prepared for submission to scientific journals and will be submitted once field trial results are available.

Most component outcomes were achieved without major difficulties owing to the good design of the project, the scientific competence and training skills of the CSIRO project

scientist Dr Penny Butcher, the dedication and hard work of the Vietnamese project scientists and technicians, and the strong support provided by the Director and senior management of the Vietnamese partner institution RCFTI and the associated Vietnamese institution, the Institute of Agricultural Genetics. We feel the project purpose has been achieved. Ongoing regular contact between CSIRO FFP and RCFTI will consolidate the skills and capacity achieved through the project. This contact will undoubtedly continue because of mutual interest in the scientific results already obtained, and in applying the capacity to additional projects.

### **3.3 Sectoral Impact**

The project has directly provided training in molecular genetics techniques for a woman scientist at the Institute of Agricultural Genetics, Vietnam, and several women technicians at RCFTI. Indirectly, the project is expected to lead to more productive forest plantations, which will be established in rural areas including those inhabited by minority communities, providing consequent economic benefits. In Vietnam, raising of forest panting stock, plantation establishment and management employ women as well as men.

### **3.4 Costs and Financing**

The project was completed within the available budget. There was no significant under-spending in components. Full acquittal is provided in Appendix A1.0.

### **3.5 Monitoring of project**

General monitoring of progress was carried out by face-to-face meetings in Australia and Vietnam, regular exchange of e-mails and telephone contact.

Monitoring of the performance of seedlings raised for the project during mid-2002 was carried out by e-mailing digital photographs from Vietnam to Australia. It became apparent that growth was much slower than anticipated, and the problem of unsuitable potting mix was diagnosed.

The issue of fencing the field trials to protect them from damage by grazing animals was raised by CSIRO in management meetings during project design in 2001. RCFTI did not consider this necessary because no damage had been noted previously. However, browsing damage did occur at Dong Ha and, in hindsight, it would have been wise to construct a fence around the trial. Fortunately, this was a subsidiary trial, and the main trial at Ba Vi, field-testing the seedlings actually fingerprinted during the project, has not been damaged.

### **3.6 Technical Assistance, Training and Capacity Building**

The project has established an independent capacity at RCFTI for their scientists to utilise molecular markers in studies of forest trees and apply the results of these studies in RCFTI's tree breeding programs. The project also established strong links between RCFTI and Vietnam's Institute of Agricultural Genetics which has been using molecular markers in crop breeding for several years. The recruitment of the Vietnamese project team was carried out by the Director of RCFTI, Dr Ha Huy Thinh, in consultation with Dr Vu Duc Quang of the Institute of Agricultural Genetics. CSIRO FFP found the Vietnamese project team to be fully satisfactory in terms of motivation, skills, prior training and gender balance. CSIRO FFP has an excellent long-term relationship with the Vietnamese partner institution RCFTI, underpinned by a Memorandum of Understanding

on scientific research collaboration. Vietnamese project staff at RCFTI are in regular (several times per month) e-mail contact with CSIRO FFP project staff regarding ongoing work by RCFTI in this area of science, and collaboration on other joint projects involving CSIRO.

The training programs carried out under the project are believed to have been fully effective in establishing a firm base of scientific and technical skills relating to use of molecular markers and their application in forest tree breeding. The results obtained in the course of the project confirm the level of competence developed by RCFTI. For example, RCFTI scientists and technicians are now trained in, and very aware of, the need for meticulous care in sampling, handling, labelling and documentation of research projects involving study of large numbers of samples. There were errors in DNA handling or mis-labelling of samples in only 6 out of over 700 DNA samples from individual seedlings analysed in the project, a very good result for a first project of this type.

A half-day Project Seminar was organised by RCFTI in March 2002, attended by the Australian project team and over 20 scientists from RCFTI and IAG. Senior managers of RCFTI reviewed the results obtained from the project, considered their implications and made plans for future projects using the capacities developed through the project. Slide presentations from the workshop are attached as Annex 1.

### **3.7 Management of Constraints, Issues, Risks and Change**

The commencement of the project in September 2001 just after the annual seed production cycle of *A. mangium* in Vietnam delayed the main scientific activity of the project until May 2001. This in turn delayed establishment of the two field trials until late 2001. The Vietnamese partner institution made a strong case for training of three Vietnamese scientists in Australia, rather than the two specified in the project design document. CSIRO FFP accommodated this request within the project budget by reducing the training period in Australia from 6 weeks to 4 weeks and accepting three trainees, two from RCFTI and one from IAG. The three trainees all had reasonable English language skills, which facilitated the training program in Australia.

A technical problem was noted in the course of raising seedlings for the project commencing in June 2002, specifically the poor quality of the potting mix used, which led to slow growth and relatively poor health of the seedlings. It was impractical to re-pot the seedlings, and this slow growth further delayed planting of the field trials. During the visit to Australia of project trainees, the composition and properties of improved potting mixes was discussed and suggestions were made for an improved mix to be used in future research trials by RCFTI.

The second field trial at Dong Ha, Central Vietnam, was damaged by cattle grazing in early 2002 shortly after planting out, with about 20% of seedlings badly affected, thus invalidating the trial objective which was to accurately compare the performance of selfed and outcrossed *A. mangium* seed. This problem had not occurred previously in research trials at Dong Ha. The problem was discussed with RCFTI and it was agreed that surplus seeds would be germinated in 2002 and a second field trial at Dong Ha would be planted out in late 2002 to replace the damaged trial, and this second trial would be fenced to prevent cattle damage.

### **3.8 Project Management**

The Lead Institution, CSIRO FFP, has maintained strong commitment to the project over the full project period. Overall management of the project was made simple because of the long working relationship and high level of trust already established between the Australian and Vietnamese project managers.

The performance of, and inputs provided by, the Vietnamese partner institution RCFTI are rated as 3 (Satisfactory overall). In the areas relating directly to capacity building for the use of molecular markers in tree breeding the rating is 4 (Fully satisfactory). In relation to the growing of seedlings for the field trial and the establishment and maintenance of the field trial at Dong Ha, the rating is 2 (Marginally Satisfactory). Lessons learned from the project will be applied in the follow-up replacement field trial at Dong Ha and in future field trials.

## **4. Performance and Outcomes**

### **4.1 Assessment of Performance Against Objectives and Design**

The project has been successful when judged against its objectives and design. The project confirmed the relevance of molecular genetics technologies for Vietnam's tree breeding programs. The capacity building and results delivered by the project enabled RCFTI to successfully attract GoV funding to develop a molecular genetics laboratory. CSIRO FFP provided advice on equipment and consumables which were necessary for molecular research. Ongoing research collaboration between CSIRO FFP and RCFTI will support the appropriate applications of the capability developed.

### **4.2 Sustainability**

The assessed rating for sustainability is 4, Fully Satisfactory. It is clear that the training and technologies introduced by the project will be used on an on-going and increasing basis by RCFTI. They are already being used on additional species by staff trained through the project. RCFTI is using its own funds to do this additional work. An indication of RCFTI's commitment is provided by the fact that in July 2002, at its own expense, RCFTI sent one of its staff to CSIRO FFP in Canberra to obtain four weeks of additional training in the use of isozyme markers, complementing that provided during the CARD project.

### **4.3 Development Impact**

The project is enhancing the capacity of Vietnamese Government agencies to deliver genetically superior trees for plantation forestry. This will enable greater levels of wood production per hectare per year from plantations, which should in turn reduce harvesting pressure on natural forests, and provide more options for land use in a country where population pressure on land resources is very high. The scientific results already obtained from the project are of great interest and practical importance. They identified the importance of ensuring a seed is only collected from seed orchards in which a high proportion of trees flower and set seed in order to avoid the negative effects of inbreeding. Results from the project will also help Vietnam to develop and manage its program of clonal forestry using acacia hybrid clones. Guidelines for seed collection from acacia seed orchards, to improve the genetic quality of seed used in plantations,

have been developed and are being applied not only in Vietnam but also in other countries which plant tropical acacias.

## **5. Conclusions**

### **5.1. Overall assessment**

The project outcomes and objectives outlined in the project design document have all either been achieved, or will be achieved by the project partners over the next two years. The adopted design has generally been appropriate. It could have been strengthened by specifying more detailed management of all technical issues relating to raising of seedlings and protection of field trials by CSIRO FFP, but this weakness did not seriously impact on the outputs and outcomes of the project. Based on their experience with this project, CSIRO FFP project staff strongly endorse the Vietnam Capacity Building for Agriculture and Rural Development Program and its continuation. The emphasis on capacity building to achieve competence in particular areas of science and rural development, and associated training provides a clear focus for collaboration between Australian and Vietnamese institutions. In our particular project, the ability to address an important research question of wide scientific and practical interest, namely the levels of selfing and outcrossing in *A. mangium* seed orchards, also ensured strong scientific interest and commitment to the project by the Australian researchers. We were also fortunate to carry out our project with a competent Vietnamese partner institution, with which we have had a long history of successful collaboration.

### **5.2. Lessons Learned**

The project has confirmed our experience from other projects, that Vietnamese scientists can rapidly learn and apply new and advanced scientific methods in the areas of forest genetics and tree breeding. It has also confirmed the Government of Vietnam's commitment to build capacity in this area, as demonstrated by GoV's financial support enabling RCFTI to build and resource a laboratory for application of molecular genetics methods.