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Contents

1 Acknowledgments

This Project built on more than 2 decades of work by many Australian and Vietnamese scientists who have contributed to a now flourishing Acacia plantation industry in Vietnam. This would not have been possible without the ongoing commitment of ACIAR and VAFS management who understood that forestry R&D requires long term commitment with continuity and that the future ultimately rests in the hands of a well trained cohort of young scientists trained with the support of the John Allwright Fellowship. Our thanks to all involved in this successful and productive collaboration.

2 Executive summary

Government of Vietnam (GoV) policy is to expand national production of plantation grown wood and tropical acacias contribute significantly, with a current area of 1.2 M ha. This includes 500,000 ha of clonal *Acacia hybrid* about half of which is managed by smallholder farmers. Production from these plantations yields feedstock for both fibre and solid wood products. Planting stock of high genetic quality, sufficient for at least 150,000 ha of plantations per year is required to maintain the acacia estate.

This Project was designed to strengthen the tree improvement capability of Vietnamese Academy of Forest Sciences (VAFS) through increasing sophistication in both strategies and technologies. There were 3 core objectives:

1. To design and implement an enhanced clonal production and deployment strategy for delivery of an ongoing stream of tested A. hybrid clones for use by tree farmers.

The staged evaluation of over 5000 hybrid seedlings and 550 promising clones of broad genetic base has positioned VAFS to deliver large economic benefits to Vietnamese growers through the deployment of a new generation of fully tested hybrid clones. Fully tested superior clones could be ready for commercial deployment by 2020. Some new clones show superior early growth to the currently planted commercial hybrids. The Project has also contributed to ongoing hybrid breeding through establishment of a new hybridising seed orchard incorporating the best VAFS pure-species clones of *A. mangium* and *A. auriculiformis*, and an information system developed to track genetic pedigrees in VAFS acacia breeding programs.

2. To refine and demonstrate deployment strategies for sexually propagated A. mangium*, to expand utilisation of seed from the elite selections planted in VAFS orchards.*

Methodology for Clonal Family Forestry (CFF) (multiplication of seedlings from tested families), was successfully developed. However in northern Vietnam the growing season was too short to deliver economically viable multiplication rates, so technology uptake will depend on VAFS identifying opportunities in southern or central Vietnam.

3. To build on a previous ACIAR Project (FST2003/002) aimed at developing new polyploid varieties with potential for improved growth rate and wood properties and reproductive sterility to contain risk of weedy invasions.

Polyploid breeding is an established approach to add new variability in economically important traits. We produced and field tested tetraploid (4x) trees of *A.mangium* and demonstrated improvements in wood properties and tree form, however growth rates are not sufficient for commercial use without further breeding. Crossing 4x trees with normal diploid (2x) trees to produce more vigorous triploid (3x) trees proved challenging but enough clones were produced to prove the concept. Follow-up field trials by VAFS have identified 2 new triploid clones with growth at least as good as 2x and they are investing in an expanded polyploid breeding program over the next 5 years.

Acacias are invasive in some ecosystems. The project has demonstrated that 3x acacia clones are highly infertile and so could be planted in ecologically sensitive areas. The Project Strategy Plan for producing highly heterozygous advanced-generation hybrid polyploid clones provides a road map for VAFS and other acacia breeders for the next decade. Tree breeding is a long term activity with value captured long after the end of any particular 5-year project.

Capacity building to allow good strategic planning and efficient program implementation is particularly important and we are happy to report real success in the training of counterpart staff on John Allwright Fellowships (JAF) at University of Tasmania (UTas) in close association with the project. The effectiveness of returning JAF scholars is exemplified by substantial GoV support to a new VAFS project that builds on Objective 3 outcomes.

3 Background

In 1998 the Government of Vietnam (GoV) adopted a program to establish an additional five million hectares of forest plantations over the years 2000 to 2010 (the 5MHRP). In 2001, the donor community and GoV jointly established the Forest Sector Support Program (FSSP) to provide coordinated assistance to this and other forestry initiatives. The World Bank and GoV have now developed the Forest Sector Development Project (FSDP) within the context of the 5MHRP and FSSP. As of 2008, Vietnam had about two million hectares of production plantations. Reliable performance of these plantations is critical to Vietnam's national economy and rural development, producing feedstock for both solid wood and fibre products. The trees are fast growing and typically harvested on rotations of less than 10 years. About half of the plantations are managed by smallholder farmers and the rest by large-scale State Forest Enterprises or Forest Companies and larger private sector companies. GoV continues to support both expansion of the production plantation area and the replanting of existing production plantations. Since tropical acacias cannot be coppiced they must be replanted after each harvest. In the course of the Project the area of acacia plantations in Vietnam expanded to a total of 1.2 M ha, including 500,000 ha of clonal *A. hybrid* plantations. Planting stock of increasing genetic quality and sufficient for at least 150,000 ha of plantations per year is required for the foreseeable future to maintain this large acacia estate.

Australian scientists were instrumental in the initial evaluation of tropical acacia species throughout SE Asia. Active collaboration with Vietnamese colleagues commenced in 1988 and has continued thereafter, with ongoing funding support from ACIAR and other Australian government organisations.

The current status of acacia breeding in Vietnam is summarised in the poster by Thinh et al (2014) and described in more detail by Harwood et al (2015). Pure-species breeding populations of *A. mangium* and *A. auriculiformis* were established in the mid-1990s, second-generation trials in the mid-2000s, and breeding is now (in 2015) about to progress to the third generation, following a strategy of recurrent selection for general combining ability in open-pollinated progeny trials that are converted to seedling seed orchards by selective thinning prior to seed collection for the next generation of breeding. Selection to date has been primarily for volume growth and improved stem form, although some weight has been given to increasing wood density.

The first acacia hybrid clones were identified and developed by VAFS in the early 1990s. These were spontaneous *A. mangium* x *A. auriculiformis* hybrids identified in *A. mangium* plantations that were established using seed collected from early plantations adjacent to *A. auriculiformis* plantings which contributed the male parentage of the hybrid genotypes. Only a very few provenances of the two parent species were involved, consequently the genetic base of these first hybrids is narrow (Kha 2001). The best clones from the first series of testing were approved for commercial deployment by Vietnam's Ministry of Agriculture and Rural Development in the late 1990s and by 2004 about 150,000 ha of clonal acacia hybrid plantations had been established.

This particular Project was focussed on a further strengthening of Vietnam's acacia tree improvement capability, especially in relation to *A. hybrid*, by introducing a new level of sophistication in both strategies and technologies. Core objectives were to:

1. Design and implement an enhanced clonal production and deployment strategy for delivery of an ongoing stream of tested *A. hybrid* clones for use by tree farmers throughout Vietnam through an integrated program of breeding, seed production management, propagation and information management strategies.
2. Refine and demonstrate deployment strategies for sexually propagated *A. mangium,* to expand utilisation of seed from the elite selections planted in the VAFS orchards.

3. To build on work started in a previous ACIAR Project (FST2003/002) aimed at development of new polyploid varieties with potential for improved growth rate, wood properties and reproductive sterility.

The Project built on substantial existing work by the Vietnamese Academy of Forest Sciences (VAFS) in the breeding of acacia species and hybrids as a contribution to the production of high value germplasm required to meet the GoV objectives for an expanded plantation estate for sawlog and fibre production. VAFS is the largest producer of improved forest tree germplasm in Vietnam and has a proven track record of effective technical support to provincial and local forest nurseries and forest extension services throughout the country. This organisation is therefore particularly well placed to manage distribution and adopting of new germplasm once proven in nationwide field trials.
Since tree breeding is a long term activity with ultimate value captured through the release of new improved varieties long after the end of any particular 5 year project it is important that strategic planning and efficient program implementation can continue whatever level of international collaboration may be appropriate in future. Capacity building through the training of counterpart staff on John Allwright Fellowships at University of Tasmania (UTas), and other activities, was therefore recognised as an essential part of the Project work program.

4 Objectives

The Project had 3 major Objectives, all aimed at adding value to Vietnam's acacia tree improvement programs by introducing a new level of sophistication in both approaches and technologies.

1. To design and implement an effective clonal production and deployment strategy to deliver clones of hybrid acacias to tree-growers throughout Vietnam, well integrated with appropriate breeding and propagation strategies. This was the core activity of the Project, reflecting both importance of this variety to the Vietnamese forest industry and the potential value which can be added by new approaches. Activities included:

- Characterisation of variation among currently available production clones of *A.hybrid* in Vietnam, including new clones not yet in commercial production.
- Production and testing of a new series of *A.hybrid* genotypes to provide a greatly expanded and genetically diverse set of clones adapted to the range of planting regions and with improved production traits (growth and form, wood quality, adaptation and pest and disease resistance).
- Specification of hybrid breeding strategies to deliver many genetically diverse elite hybrid family combinations for ongoing clonal selection and evaluation
- Development of a new reproductively isolated clonal hybridizing orchard with focus on its optimal location and management for production of selected hybrid family combinations
- Design and implementation of a clonal deployment strategy that optimally balances delivery of genetic gains and minimisation of risk.

2. To develop improved genetic deployment strategies for the sexually propagated *A. mangium* and demonstrate their practicability. This sub-project was aimed at assisting VAFS to deliver improved value from their existing breeding and seed orchard programs. Activities included:

- Design of breeding and propagation strategies optimally interfaced with the deployment strategies appropriate to the biology of this species
- Refinement of sexual and asexual propagation protocols to optimise effective and efficient delivery of propagules in required numbers
- Demonstration of intensively managed clonal seed orchards, appropriate to all *Acacia* species, and efficient nursery systems for deployment through Clonal Family Forestry (CFF).

3. To continue development of promising new polyploid varieties of *A. mangium* and *A. hybrid*. This was a research phase activity with potential medium to long term benefits to Vietnamese and international growers and forest industries. The work program built on experience gained through earlier investment by ACIAR and VAFS. Activities included:

- Further assessment of the potential role of polyploids in Vietnamese breeding and clonal programs through production and field testing of polyploids derived from elite hybrid material.
- Development of means of efficiently mass-producing polyploid seeds and clones
- Development of a long term breeding strategy with integration to the mainstream *A.hybrid* improvement program

5 Methodology

Methodologies are discussed in relation to each of the 3 main Objectives

Objective 1 – Development of new hybrid clones:

This work was carried out entirely in Vietnam under the management of Mr Do Huu Son and with support from nursery staff at Ba Vi and field station staff in central and southern Vietnam. Dr C Harwood (CSIRO) advised and supported trial analyses.

Review of existing genetic base of commercial hybrid clones. The ACIAR project reviewed acacia hybrid development up to 2010 and the performance of the hybrid clones commercially deployed in Vietnam (Kha et al. 2012).

Although training in NIR (Near InfraRed Spectrometer) use was provided by the project, the Institute of Forest Tree Improvement and Biotechnology (IFTIB) was not able to purchase an NIR system for screening of pulp yield.

There are several reasons why it is necessary to expand the numbers and breadth of genetic base of hybrid clones available for Vietnam:

- Identify clones with superior tolerance to diseases such as *Ceratocystis* which have already had severe impacts on acacia plantations in other SE Asian countries and are known to be present in Vietnam
- Identify clones with superior adaptability to particular site conditions (wind-firmness, drought tolerance) so as to extend the planting range for *A. hybrid* in Vietnam
- Identify clones with improved stem and branch form and wood properties, more suitable for veneer log and sawlog production than current commercial clones

The project therefore implemented several strategies to expand the numbers of hybrid clones available for planting in Vietnam

Identifying and screening new hybrid clones using existing OP seed sources. The project implemented a short-term measure to greatly expand the number of candidate clones for evaluation (Do Son et al (2014), Harwood et al (2015)). Bulk and individual-family seedlots of *A. mangium* and *A. auriculiformis* were obtained at locations where genetically diverse, selected populations of the two species had been established in adjacent stands. Seedlings from these seedlots were raised in the nursery and hybrid seedlings identified on their morphology; the proportion of apparent hybrid individuals identified was about 1%, although this varied according to seed source. Four field trials of hybrid seedlings along with commercial hybrid clones as controls were then established at 4 locations across Vietnam in years 2009-10, testing in total over 5,000 putative hybrid candidate genotypes. At age 2 years, these trials were assessed and the best (in terms of vigour, and with acceptable stem form) 5% or so candidates in each trial were identified for capture as clones, multiplication and clonal testing. Four clone screening trials across Vietnam were established in years 2011-12, testing a total of 210 candidate clones identified in this way. Hybrid candidates from both *A. mangium* and *A. auriculiformis* mother trees were selected. The trials also tested an additional 340 candidate clones captured (independently of the Project) by IFTIB from second-generation progeny trials established in their pure-species breeding programs, and included commercial acacia hybrid clones and seedling controls of the two parent species. These trials were in turn assessed at age 2 years for growth, stem and branch form and, in some trials, wood basic density and stiffness. Phyllode and stem morphology, relative to the two parent species, was also scored. This scoring, subsequently supported by molecular genetic analysis, suggested that many of the candidate hybrid clones, particularly those selected from IFTIB pure-species progeny trials surrounded by acacia hybrid plantations, may be complex hybrids such as back-crosses arising from pollination of pure-species mothers by

pollen from hybrid trees. This finding raised important issues for both hybrid breeding and pure-species breeding. The most promising clones are currently being identified and will be re-tested in second-stage clone proving trials at several locations in Vietnam, to be planted in 2016.

Establishing an elite hybridizing seed orchard. A longer-term component of the project's hybrid breeding strategy was to establish an elite hybridizing orchard, comprising 10 selected superior clones of each parent species, identified in the second-generation pure-species progeny trials. Grafted ramets of the 20 clones are set out in intimate mixture (single-tree plot layout) and the orchard is isolated as far as possible from acacia pollen sources that could contaminate the orchard. The aim is to maximise the number of favourable hybrid combinations derived from open-pollination between selected pure *A. mangium* and *A. auriculiformis* genotypes. Controlled pollinations may also be undertaken. The orchard will also produce elite pure-species seed that can contribute to pure-species breeding programs. Because of the limited number of pollen parents in the orchard, recovery of male pedigree of OP seed through molecular marker analysis should be possible. This orchard was planted at VAFS Phu Yan station in Dong Nai Province, south Vietnam, in September 2013 and seed production commenced in April 2015.

Developing hybrid clones from seeds produced by control-pollination. A third avenue for production of new hybrid candidate genotypes is controlled pollination (CP). Over 1000 CP diploid hybrid seeds were produced from several interspecific crosses among selected diploid clones of *A. mangium* and *A. auriculiformis* in the Bau Bang hybridizing orchard as part of the polyploid breeding activities (see Objective 3). These seeds are being progressively germinated and screened in hybrid seedling trials as an additional Project activity.

Developing polyploid hybrid clones. This option is described in more detail under Objective 3. It is important to integrate hybrid and polyploid breeding with pure-species breeding; this will be facilitated through the newly developed information management system..

Developing an information management system. To monitor genetic diversity in successive breeding generations and to correctly analyse advanced-generation progeny trials to accurately determine genetic parameters and manage inbreeding (which is known to lead to reduced vigour in these species), it is necessary to track pedigrees and genetic relatedness in the main (open-pollinated) breeding populations, OP hybridizing seed orchards and CP breeding programs. After investigating various alternatives, a simple information management system was developed using Microsoft Excel software, which is familiar to Project partners. At Project completion, the system had been fully developed for IFTIB's pure-species breeding populations *A. auriculiformis*, *A. mangium* and *A. crassicarpa*, and was being extended to cover all acacia hybrid clones and polyploid breeding populations of acacia hybrid and pure species. The system is outlined in Appendix 1

Objective 2 – To refine and demonstrate new deployment strategies for sexually propagated *A. mangium*.

Again the work was conducted wholly in Vietnam by IFTIB staff following an initial visit to RAPP in Indonesia and with the support of Australian Project Scientist Mr P Warburton

As a pure species, *Acacia mangium* is less well-suited to clonal forestry than are *A. auriculiformis* and acacia hybrid, because of ontogenetic ageing effects in the clonal hedge plants from which cuttings for rooting and field planting are harvested. Attempts to develop clonal forestry with pure-species *A. mangium* and *A. crassicarpa* in Vietnam during the 1990s, in parallel with acacia hybrid, were not successful. In contrast, a

number of selected *A. auriculiformis* clones have been developed and tested and licensed for commercial production by MARD.

Clonal Family Forestry (CFF) is the multiplication of seedlings from superior family seedlots using vegetative propagation methods, without retention of individual clone identities (White et al 2007). This reduces the cost and time to deployment relative to conventional clonal forestry, because the selection and testing of individual clones and the need to retain their identities are eliminated. Genetic gain in deployment is achieved by applying CFF to outstanding family seedlots, whose superiority has been confirmed by progeny testing. CFF bulks up the numbers of planting stock of these families relative to that obtainable if the seeds from these families were propagated directly in a seedling nursery. This deployment system is well suited to species such as *A. mangium* which rapidly lose their capacity for vegetative propagation as the seedlings age. Individual *A. mangium* hedge plants can then be discarded as soon as ageing effects become apparent. This contrasts with conventional clonal forestry where it is necessary to maintain and multiply individual clones for 10 or more years while their performance is evaluated in clone trials prior to mass clonal deployment.

The feasibility of CFF with *A. mangium* was originally reported by (Wong & Haines 1992) and operational CFF deployment programs exist today in Sumatra, Indonesia to multiply genetically improved family seedlots collected from known superior trees in *A. mangium* and *A.crassicarpa* seed orchards (Wong 2010).

The success of CFF is dependent upon maximising the production capability of the cutting production system and integrating this production with the seasonal requirements for planting stock for field planting of plantations. The Project therefore evaluated the feasibility of using CFF for mass-deployment of superior seed families of *A. mangium* in Vietnam, using systems adopted from those used in Sumatra. A series of trials was conducted at IFTIB's research station at Ba Vi, Hanoi Province, northern Vietnam. Factors tested included hedge plant nutrition (three commercially available liquid complete fertilizer systems were tested), establishment time for hedge plants and harvesting schedules for cuttings. Cutting production and rooting of harvested cuttings were tracked over three successive growing seasons, so that multiplication rates could be established. High rates of cutting production and rooting of harvested cuttings (generally over 80%) were achieved during the summer months, but both declined in the autumn and cutting production was not possible in the winter months under the prevailing conditions of low temperatures and light intensity.

A field trial comparing the performance of CFF rooted cuttings with seedlings from the same 10 *A. mangium* families used in the CFF study was established at Ba Vi in October 2012.

It was intended to evaluate CFF under commercial nursery conditions and train commercial operators in this technology. A pilot system was developed with IFTIB support in a nursery at Dong Ha, Quang Tri Province, central Vietnam, and produced *A. crassicarpa* cuttings for field planting for several months in 2013, but the nursery had to close down because of highway realignment before substantial production data could be collected.

This Objective also addressed efficient seed production in acacia seed orchards. Review of seed harvest data from IFTIB's existing seed orchards confirmed that orchards established on deep soils with good drainage in SE Vietnam generally produced heavy seed yields from an early age making this region the best location (preferred over central and northern Vietnam) for new seed orchards. Tree spacing and crown management protocols developed at the polyploid hybridizing orchard at Ba Bang in SE Vietnam were developed over several years and provided valuable guidance for the new hybridizing orchard subsequently established at Tan Lap.

Objective 3 – To continue development and evaluation of promising new polyploid varieties of *A. mangium* and *A. hybrid*.

The work program was shared between Vietnam and Australia (Dr Griffin, Ms Harbard and Mrs Price at UTasmania) with fibre and pulp analyses conducted in South Africa by SAPPI and at the laboratory of Norske Skog in Tasmania. New polyploid genotypes were produced at U Tasmania and transferred to Vietnam as unrooted cuttings or in tissue culture for multiplication and field testing under the control of Mr Do Huu Son and Dr Nghiem Quynh Chi; flow cytometry was conducted at UTas until the final stage of the Project in which we supplied a new instrument to VAFS together with appropriate training. Dr Chi also managed production of open and controlled pollinated seed production. The original plan envisaged parallel field testing in Queensland. A trial was established at Tully and had reached flowering age when it was destroyed by Cyclone Yasi. Rather than repeat we focussed remaining effort entirely in Vietnam.

Both laboratory and field based activities were required in order to produce and test polyploid lines for eventual commercial use. Since no polyploids of these tropical acacias had been grown before their biology was unknown and needed to be elucidated as the project progressed and that knowledge then used to adapt the breeding strategy as problems and opportunities were encountered. As a result there were a number of modifications to the original Activities as defined in the Project document. Although the work of John Allwright students associated with the Project was not formally part of the work program, the studies of Mr Tran Duc Vuong M.Sc. and Dr Nghiem Quynh Chi Ph.D. on the breeding system and reproductive biology of the original neo-tetraploid *A.mangium* were fundamental to our understanding and greatly influenced the strategy plans presented at the end of Project. Methodologies for their studies are reported in key publications Griffin et al (2012), Nghiem et al (2011), Nghiem et al (2013), Nghiem et al (2015 submitted) and are not presented here.

The project is based on a set of tetraploid (4x) clones “Shell Clones” produced by colchicine treatment of seeds of *A.mangium* by Shell Forestry Ltd in UK and subsequently gifted to Prof Kha of VAFS who propagated them and established in an orchard at Bau Bang southern Vietnam in 2003. This work was the subject of ACIAR Project FST2003-002. The work of Mr Vuong and Dr Chi was conducted in this orchard and seeds were used as a basis for the studies of open pollinated seedling growth and of frequency of triploid (3x) production which were part of the work program in the current project.
The Shell Clones were also propagated by the team at U Tasmania and along with new 4x genotypes produced in UTas were planted in the field trial at Tully Queensland for observation of the morphological characteristics reported in (Harbard et al 2012).

In presenting this overview or Methodologies we have deviated from the organisation of Activities as tabulated in Section 6 to avoid repetition, since there were common approaches applied to study of different types of genetic material.

Characteristics of neo-tetraploid genotypes in comparison with normal diploids:
A common observation is that in plants with a doubled chromosome complement the cells are larger than in progenitor diploids, a phenomenon known as gigantism. This can result in changes to the morphology and growth of the plant and affect physiology and biochemistry. Such changes may be positive or negative in terms of crop production so it was of interest to document all observable differences as the 4x trees grew and began to flower.

Traits assessed included phyllode, spike and flower dimensions, size and frequency of stomata; bark thickness and tannin content; fibre dimensions and pulp properties; flowering traits; and physiological response to water stress.

Morphological studies were conducted on trees in the Queensland field trial, in Vietnam and on propagules raised in the glasshouse at UTas. Light microscopy was used at UTas for stomatal observations and for determining polyad dimensions.
For the pulping study billets were taken from 2x and 4x trees in the Bau Bang orchard, chipped in Hanoi and freighted to Pretoria South Africa where tests were conducted at the laboratory of a collaborating company SAPPI. Duplicate 1kg wood samples and Kraft pulp yield at Kappa 20 was determined; pulps were refined in a PFI mill to make handsheets for assessment of physical properties; fibre strength properties were determined with a Pulmac Z –Span 3000 instrument and fibre dimensions quantified by Techpap Morfi Fibre Analyser. Properties of the acacia pulps were then compared and contrasted with published characteristics of eucalypt pulps to give a perspective on possible commercial utility. Additional assays of fibre dimensions were conducted by UTas using the Metso FiberLab instrument owned by Norske Skog.

An assay for content of phenolic extractives from bark was developed at UTas. CSIRO Land and Water in Hobart conducted a glasshouse experiment comparing response of 2x and 4x lines of hybrid clones to water stress.

Production of new 4x lines from Seeds or Clones: Any long term breeding program is dependent upon access to a large and genetically diverse population to interbreed and select. An important positioning activity for the Project was therefore to produce new 4x lines of *A.mangium* to supplement the Shell Clones. This work was carried out at UTas initially with seeds and then with tissue cultures of commercial hybrid clones sent from Vietnam. Protocols which optimised production of stable 4x genotypes were developed. These were used to generate lines which were transferred to Vietnam for multiplication and field evaluation. Since we had the capacity we also produced sets of 4x plants from seeds of *A.auriculiformis* and *A.crassicarpa.* These give VAFS the option of producing novel polyploid hybrid lines once they commence flowering. The *in vitro* protocol for colchicine treatment of commercial hybrid clones is particularly important for the future breeding strategy since it offers fast track access to superior genetic combinations.

Verification of Ploidy: A complication of colchicine treatment is that it only doubles cells which are actively dividing at the time of application. A meristem consists of thousands of cells some of which may remain diploid and some doubled. This produces a plant termed a mixoploid. Depending on which cells were induced different tissues in the resulting plant may be 2x or 4x.This is a particular problem where a plant is multiplied by cuttings each of which derive from a different axillary meristems. We are looking for plants which are stable 4x in both somatic and reproductive tissues so it is essential to make more than one screen as the plant matures. Our protocol is to use a Partec Cyflow Ploidy Analyser to assay samples of tissue taken from phyllodes on at least 2 occasions before a plant is cleared for transfer to Vietnam (Price et al 2014). Following multiplication in Vietnam it is necessary to once again check the ploidy. The importance is emphasised by the fact that we found only 16 of the 38 original Shell Clones were stable 4x in respect to all traits at flowering maturity. A version of this instrument has now been purchased for VAFS from Project funds and they are equipped to use the technique for their future work program.

For breeding it is ploidy in the reproductive structures which is most important. By measuring floral and polyad dimensions under the microscope it is possible to classify a plant as 2x or 4x. This was done for trees in the Bau Bang orchard and seed was only collected from trees confirmed 4x.

Transfer multiplication and field establishment in Vietnam:
The distribution of workload between Tasmania and Vietnam presented some unique challenges. For quarantine and logistical reasons we could not root propagules in soil medium in Hobart and send to Vietnam. We needed to take cuttings and transfer these to as soon as possible for rooting and further multiplication by VAFS staff. After several

abortive attempts to export using a courier it was found that the best way was to wrap the cuttings in wet cotton wool or paper, place in an cool box and take as hand luggage when Project staff were going to Vietnam. Appropriate import documentation was provided by VAFS on each occasion. Nursery staff met the plane in HCM city and had the material set in propagation beds within 24 hours of excision. This worked well on numerous occasions. For the 4x lines of hybrid clones produced *in vitro* the process was simpler as cultures could be hand carried and used for multiplication and weaning of plantlets for field testing at the VAFS laboratory in Hanoi.

VAFS were responsible for finding suitable field trial sites and for planting trials according to design specifications agreed with Australian Project staff. Good management in particular weed control was essential and VAFS was responsible for this together with collection of data as the trials matured. Data was transferred to Australia and analysed by Dr C Harwood of CSIRO.

Triploids: The original plan anticipated that the Bau Bang 2x / 4x orchard of Shell Clones would produce triploid (3x) progeny by open pollinated crossing between the cytotypes. Seed was collected from as many clones as possible and raised in a glasshouse at UTas where progeny were screened with the Ploidy Analyser. No 3x plants were found among the sample of progeny from 4x parents (although we subsequently found 1 in a progeny trial of seed from one clone and the huge growth superiority of that tree compared with 4x siblings provided impetus to the triploid breeding program both within the Project and subsequent GoV funded research). The screen of glasshouse grown progeny from Bau Bang orchard was then focussed on 2x mothers and at low frequency 3x plants were detected. From their morphology these were not hybrids and we inferred that they derived from fusion of an unreduced gamete from a diploid with a normal haploid gamete. This is a phenomenon reported from many plant species in the literature.
Although we did not derive 3x seed by open pollinated intercytotype crossing we thought that we might have more success by hand pollinated crossing between 2x and 4x trees. To prepare for this work a breeding arboretum was planted with grafts of some of the Shell Clones and 2x controls of both *A.mangium* and *A.auriculiformis* from the Bau Bang orchard. Crown management protocols were developed to keep the flowering zone of the trees within accessible reach of the ground as it is very expensive and difficult to manage pollinations on large trees. Pollination methods were developed based on experience of Project staff in Indonesia and Vietnam and we are confident that the methodology is as good as possible.

One of the key aims of triploid production is that they are expected to be effectively infertile (because the 3 sets of chromosomes cannot pair during meiosis). The 3x clones which were produced or discussed were all propagated into a breeding arboretum for observation of inflorescence and floret development and subsequent seed set. The trees had only just begun to flower at the end of the Project but Dr Chi has been able to continue the observations and as of May 2015 we have sufficient data to draw publishable conclusions. The methodology used in the field and for microscopic examination of pollen tube growth are as used by Dr Chi in her Ph.D. studies. The first seed produced from the 3x tree in the 4x progeny field trial was shared between Hanoi and Hobart. Studies of seed weight, imbibation and germination of seeds, and ploidy were conducted in Hobart allowing conclusions about the effective fertility of the parent.

Strategy Plan Development: An important deliverable at the end of the Project was an outline Strategy Plan to take the polyploid breeding program forward (Griffin et al 2015). This was based on an extensive literature review of experience in crop breeding and of the few tree taxa where this approach has been utilised, taking our new knowledge of the reproductive biology and production traits of acacia into account.

6 Achievements against activities and outputs/milestones (Activities added through the course of the Project in RED)

Objective 1: To design and implement an improved clonal production and deployment strategy to deliver clones of hybrid acacias to farmers throughout Vietnam.

no.	activity	outputs/ milestones	Completion Date	Comments
1.1.	Enhanced utilisation of existing clones		Jan 2012	Good performance of existing commercial clones established and some more recently developed clones in genetic trials where they were tested in different regions of Vietnam.
1.1.1	Review of existing trial data in relation to major growing regions in Vietnam	Cross site analyses completed	April 2011	Completed, published and used to help confirm good growth performance of existing commercial clones some new clones. Effective evaluation of basic density and pulp yield of existing hybrid clones was not achieved, although pilodyn revealed significant differences in density
1.1.2	Identification of any major gaps in testing program for existing clones	Recommendations for additional trial plantings by VAFS	Dec 2011	Review completed and recommendations made to expand trial network, south-central Vietnam identified as a region where additional testing is warranted
1.1.3	Develop capability for NIR prediction of Kraft pulp yield (KPY)	VAFS staff trained in Australia.	August 2011	VAFS staff trained in sampling and calibration of Near Infra Red analysis in Hobart
1.1.4	Assess existing commercial hybrid clones for Kraft pulp yield	Data available and integrated with analysis of other economic traits	Not achieved	Capacity developed but NIR technology not in place at VAFS to evaluate breeding populations
1.1.5	Review options for information management system	Action plan to acquire/modify most fit for purpose system	September 2014	MS Access learned by VAFS, but Excel finally considered more accessible to all. Basic system designed and developed
1.1.6	Develop information management system for hybrid breeding and capable of extension to pure species	System developed, documented and in use by VAFS staff	June 2015	System of pedigree tracking was developed using Microsoft Excel. A comprehensive pedigree management database and accompanying user notes were completed in mid-2015 for IFTIB's pure-species breeding population *A. auriculiformis*, *A. crassicarpa* and *A. mangium*. Pedigree

	programs			database yet to cover hybrid clones and polyploid genotypes. IFTIB staff not yet fully trained in pedigree system use.
1.1.7	Updated prescription of best clones for planting in each region	Recommendations to MARD	April 2011	All existing clones ranked in individual trials. New clones have not been tested in clone proving trials throughout Vietnam and project staff will continue this work beyond the extension period
1.2	Prescription of strategy and methodologies for long term improvement of the A. hybrid variety		March 2014	Long term breeding strategy documented and presented at IUFRO meeting, effectiveness of different hybrid selection strategies and influence of maternal parental species quantified
1.2.2	Review of multi-trait breeding objectives and appropriate economic weights	Review and associated modelling complete	April 2011	Review of industry requirements and price differentials completed. A single set of economic weights could not be estimated as different sets of stakeholders (small growers, large growers, chip exporters, local pulp producers and solid wood processors have clearly differing improvement objectives; no single objective will be applicable.
1.2.3	Review information from 1.1, incorporate plans for 1.3 and 1.4 activities and foreshadow advanced generation procedures.	Draft document written	March 2014	Completed (see 1.2.4 below)
1.2.4	Review and update draft on basis of experienced gained in the Project	Final document prepared and published	March 2014	Current acacia hybrid breeding strategy summarised in Du Huu Son et al (2014) poster at “Acacia 2014” IUFRO conference Hue. Aspects of strategy presented in more detail by Harwood et al. (2015)
1.3	Identification of new hybrid clones from existing seed sources			500 new hybrid clones evaluated in 4 clone trials, in comparison with existing commercial hybrid clones. Two-year assessments of 4 clone trials completed in December 2013. Results from Ba Vi and Dong Ha clones trials presented in posters at IUFRO conference, March 2014. Full analysis of two-year results will be undertaken by Mr Do Huu Son as part of his PhD thesis, with assistance from Dr Harwood..
1.3.1	Select and raise best Am and Aa OP families from VAFS hybridizing orchards, identify hybrid progeny	At least 5000 hybrid seedlings identified and raised for field trials	Sept 2010.	Hybrid seedling trials established at 5 locations: Tuyen Quang, Ba Vi, Dong Ha and Bau Bang (2 trials)
1.3.2	Locate and prepare 3 field trial sites	Trial sites in n, c and s Vietnam for planting 1.5 ha field trials	July 2012	Established four trials – one more than requested
1.3.3	Plant field trials of hybrid seedlings and controls	Three 1.5 ha field trials planted, family	Aug 2012	By August 2010, 4 hybrid seedling trials in north, central and south Vietnam were successfully established with 5000 genotypes. Beyond what was required

		identities of hybrid seedlings maintained		within the project, VAFS established 2015 field trials testing several hundred hybrid F1 genotypes produced by controlled pollination among superior Aa-2x and Am-2x clones in the hybridizing orchard at Bau Bang
1.3.4	18-month assessment of hybrid seedling field trials	Field trials assessed, data analysed	March 2012	Field trials evaluated in 2011-2012 and selection decisions taken
1.3.5	Clone best hybrid genotypes	At least 100 top hybrid genotypes clonally propagated	April 2012	210 selections were captured and cloned from the seedling trials. 340 additional hybrid candidate clones were selected from IFTIB second-generation pure-species progeny trials
1.3.6	Locate and prepare 3 field trial sites for clone screening trials		June 2012	Planted in Northern (Bau Bang, Bac Giang), Central (Dong Ha) and Southern (Bau Bang) Vietnam
1.3.7	Plant 3 clone screening trials	Test cloned hybrids and controls in north, central and south Vietnam	Sept 2012	4 clone screening trials planted testing a total of 550 clones and commercial controls in Northern (Bau Bang, Bac Giang), Central (Dong Ha) and Southern (Bau Bang) Vietnam
1.3.8	18-month assessment of clone screening trials	Hybrid clonal selections ranked in screening trials and gains estimated. Presentation at wrap-up workshop	Sept 2014	2-year results identified some new clones with early growth superior to that of commercial hybrid control clones
1.4	Positioning for production of next generation hybrids from superior parental selections			Achieved
1.4.1	Review VAFS pure species trial info to select best parents for new hybridizing orchard	Best 10 (minimum) Am and Aa parents identified for hybrid breeding	December 2012	Ten selections made in second- generation progeny trials of Aa and of Am following 4-year assessments.
1.4.2	Graft best Am and Aa parents for new CSO	Selections of Am and Aa grafted in sufficient numbers for CSO	March 2013	Completed
1.4.3	Design field layout suitable for intensive crown and seed production	Prescription prepared, including experimental treatments where	March 2013	Site secured and orchard designed for maximizing flowering, outcrossing and interspecific outcrossing

	management practices	optimum requires definition		
1.4.4	Select and prepare CSO site and plant	CSO established	Sept 2013	CSO established at Phu Tan field station in September 2013
1.4.5	Manage CSO for maximum flowering and seed production	Experimental treatments to maximise seed production evaluated	April 2015	Prescription for management understood by field staff.
1.4.6	Seed collected after first mass flowering	Seed collected with female parental identities retained	Not yet completed	Flowering being monitored. First seed collection from early-flowering trees undertaken in April 2015.

Objective 2: To refine genetic deployment strategies for sexually propagated A. mangium and demonstrate practicability.

no.	activity	outputs/ milestones	Completion Date	Comments
2.1	Review preconditions for clonal family forestry (CFF)			Achieved
2.1.1	Visit of Project staff to Indonesia	Report on methodologies and applicability to Vietnamese context	Sept 2009	Visit successful and information used as basis for planning of project work in Vietnam
2.1.2.	Identify 5 elite Am op families from VAFS seed orchards with outstanding field performance, uniformity and good rooting ability	Ten families identified following rooting screen trials in VAFS nursery	March 2010	Completed
2.1.3	Identify nursery for CFF research & later demonstration	Nursery identified by VAFS	June 2010	Cam Quy research nursery at VAFS Ba Vi station used
2.2	Protocol development and evaluation			Documented in detailed technical report to ACIAR and poster presented at “Acacia 2014” IUFRO conference

2.2.1	Design and install CFF nursery propagation system	CFF system that can operate in Vietnamese conditions designed	June 2010	CFF system designed and installed using local materials and nutrient solutions
2.2.2	Experimental program to evaluate hedge plant management options and multiplication rates	Hedge plant management prescriptions optimized	Nov 2012	Experiments carried out to evaluate key productivity factors
2.2.3	Establish field trials testing CFF	At least 2 field trials (each 1 ha) comparing cuttings and seedlings of elite families + commercial Am controls	One trial of 1 ha established October 2012 at Ba Vi, northern Vietnam	Trial demonstrated no difference in growth or survival of cuttings versus seedlings at age 2 years
2.2.4	Review of technical issues and cost: benefit analyses	Recommendations following workshopping	September 2014	Detailed Project technical report, published on Project website in April 2014. Cost:benefit analysis not completed because multiplication rates in northern Vietnam clearly insufficient to make CFF feasible there. Recommendation to apply other methods in northern Vietnam and evaluate CFF in central and southern Vietnam
2.3	Technology transfer (timeline dependent on progress with 2.2 and complexity of decision re general utility)			Not progressed as planned because of 2.2.4 conclusion
2.3.1	Systematic review and contact with organisations which will be included in extension program	Extension plan prepared and agreement reached with all participants	December 2012	VAFS contacts in industrial nurseries assessed partner viability for technology uptake
2.3.2	In-nursery training conducted for staff of candidate nurseries	Training course written materials produced, training courses run	March 2014	VAFS staff provided training for commercial nursery staff 2012-3. Pilot operation of CFF using superior *A. crassicarpa* families trialled in commercial nursery at Phong Dien - Thua Thien, Hue, central Vietnam. Nursery closure caused inability to collect data, multi-nursery trials would be useful to understand what environmental variables most impact propagation efficiencies
2.3.3	CFF manual produced	Comprehensive illustrated CFF manual in Vietnamese and English produced	Not achieved	Demand for manual was judged insufficient as technology is unproved; other CFF approaches involving tissue culture and isolated orchards currently being evaluated by VAFS may be preferable. Manual could be produced based on technical details included in Project Technical Report

Objective 3: Continue development and evaluation of promising new polyploid varieties of A. mangium and its hybrid

no.	activity	outputs/ milestones	Date completed	Comments
3.1	Produce strategic plan for polyploidy breeding	Written plan	December 2014	Project strategy completed, broader strategy outlined Griffin et al 2015. More detailed plans made in consultation with VAFS during extension year. Recommend major review on 3 yr cycle based on information from Dr Chi's research
3.1.1	Model expectations from different polyploidy breeding strategies	Different polyploidy breeding objectives modelled	Not achieved	Quantitative modelling not undertaken as insufficient data available.
3.2	Field testing of 3x plants in Vietnam and N Qld	Field trials in the ground and assessment begun	2011-2015	11 new 3x genotypes being multiplied for field testing in 2016. To extend beyond life of the project.
3.2.1	3x plants identified and material prepared for planting	3x plants identified and raised in Hobart.,Cuttiings transferred to Vietnam.	2013	Proof of concept achieved. VASF positioned to continue search for 3x from unreduced gametes
3.2.2	Trial sites in Vietnam and N Qld prepared and trials planted	Trials planted with 2x controls	February 2011/14	Queensland trial lost in cyclone Yasi 2011. Work continued in Vietnam. Ongoing management essential for VAFS
3.2.3	3-year assessment of field trials	Data collected and analysed	Growth assessment not achieved. Data re infertility collected 2014/2015	Loss of Qld trial and late establishment of Vietnam trial means VAFS need to complete the growth assessment. Fertility data reported "Acacia 2014" IUFRO Conference. Now sufficient for high quality peer reviewed paper
3.3	S1 4x Am plants established in breeding orchard	Seed collected from 4x trees. Raised in nursery and established in trial in Vietnam. Growth data analysed	2009- December 2014	Evaluation completed and reported in Griffin et al 2015.
3.3.1	Breeding orchard of 4x OP families established in good	Selections made from 2 best families , grafted and	August 2010 - ongoing	Grafts growing well at Phu Tan field station but not yet flowered. Breeding system study in Mr Le Son Ph.D program; Dr Chi to continue crossing in her 3x

	flowering location in Vietnam	established in Breeding arboretum		development program
3.3.2	OP seed collection from 4x orchard for testing of parental lines	Seed collected and seedlings in next generation trial.	Not achieved as not yet flowering	Resource for Dr Chi program in future. Fertility and breeding system relative to neo-tetraploids will have important implications for breeding strategy
3.4	Production of pure outcrossed 4x Am lines, and 3x hybrids (Am x Au)	Clones flowering. Controlled crosses made.	Not achieved within term of project. In Dr Chi's plans for her MARD project	Breakthrough by Dr Chi in successful capture of hybrid 3x seedlings by tissue culture has positioned for further development of more clones for field testing
3.4.1	Crossing using pollen from Bau Bang Am tetraploids to produce outcrossed 4x F1 plants and hybrids with diploid Aa	Controlled crossed lines successfully produced.	Not achieved in term of project	Focus moved to production of 3x hybrid clones which will be more easy to propagate than pure Am 3x or 4x
3.4.2	Screening of progeny and propagation of selected plants for field evaluation	Polyploid progeny identified and tested in field trials against appropriate controls	Not achieved within term of project	Core activity in Dr Chi's MARD Project.
3.5	Produce new 4x Am plants from elite 2x families	Laboratory work executed in Vietnam following tech transfer of FST2003/002 protocols	March 2013	Transferred 4x clones to VAFS: 58 *A mangium* clones , 28 clones of *A. crassicarpa* and 45 of *A. auriculiformis* were an additional effort by UTas staff not in the original proposal. Colchicine inductions on both acacias and eucalypts now being done by VAFS
3.5.2	New 4x Am plants produced and incorporated into 4x breeding populations	4x individuals induced from seed of elite Am 2x families (refer to activity 1.3.1)	June 2011	New clones transferred to Vietnam as unrooted cuttings and 45 now established in field at Phu Binh. Expected to flower in 2016. Part of Dr Chi MARD project
3.6	*In vitro* colchicine induction of operational hybrid clones	Lines transferred from Vietnam to Hobart. 4x lines induced multiplied and returned to Vietnam	2009-2013	5 2x hybrid clones transferred from Hanoi to Hobart where 4x lines were successfully created with colchicine and returned to VAFS.
3.6.1	Method optimisation at UTas	Methods optimised using commercial	2009	Successful. See poster from "Acacia 2014" IUFRO conference

		clones from Vietnam		
3.6.2	Production of 4x hybrid lines for transfer to Vietnam	Polyploidy lines produced in Hobart and sent to Vietnam for bulk up	February 2010	32 4x lines from 4 2x operational hybrid clones successfully transferred
3.6.3	Field trials established in Vietnam and N Queensland	Field trial established to comparing polyploid hybrid clones with original 2x lines	August 2011 (Vietnam only..Queensland program aborted following loss of collaborator due to Cyclone Yasi).	source of information/ new clones for implementation of advanced generation polyploid breeding strategy
3.6.4	2-year evaluation of field trials	Growth and survival data collected and analysed	August 2013 (and ongoing)	Growth and form assessed and reported in Griffin et al 2015.
3.6.5	Crossing of hybrid lines and 4x X 2x variants of clones		May 2014/15 (OP seed only not CP)	OP seed collected May 2014 and again 2015. Part of Mr Le Son Ph.D. program using new molecular markers
3.7	Fibre length evaluation of 2x/4x Am		2012	Lab studies of fibres at Norske Skog New Norfolk. Pulping by SAPPI South Africa and solid wood properties by VAFS. Peer reviewed publication.
3.7.1	Review information from previous ACIAR project	Status report/ recommendations on future sampling with assistance of CSIRO expert	2008	Advice from G Downes used in planning project
3.7.2	Acquire new wood samples from large 2x and 4x trees, measure fibre length, analyse & report results	Wood/ pulp analyses completed by collaborating lab (SAPPI South Africa)	2011	Fibre length of 8-year-old wood from 4x Am clones confirmed as longer than 2x with superior pulp properties and wood stiffness. Fibre dimensions of 5 year old wood from 3x clone X01 was determined in extension year
3.8	Assessment and recommendations regarding future of polyploid breeding and integration with main-stream 2x improvement	Review paper prepared and workshopped	December 2014	Strategy outlined in ACIAR meeting and to be published in Southern Forests. More detailed plans in consultation with VAFS (see1.2.4 and 3.1 above)
3.9	Assess fibre characteristics of young 4x/2x progeny	Confirmation that that the differences observed in 3.7 are	June 2012	Fibre length of 2 year old 4x selfed progeny confirmed as longer than 2x Data completed and prepared for publication. Trial is important source of 4x fibre for scale up pulping studies

		heritable		
3.10	Bark properties of 4x and 2x trees	Review of methodologies for assay of tannin extractives from bark. Evaluate differences between 2x and 4x	September 2013	Assay methodology in Technical Report on website. Indication that 4x may have higher extractives but any further work dependent in interest from an industry partner
3.11	Alternative methods for ploidy classification	Comparison of flow method with others (NIR, chloroplast fluorescence, chromosomes, guard cell size)	Formal study not completed as clear that if a flow cytometer is available then this is the most effective method of processing large sample numbers.	Options discussed in various publications. VAFS have instrument and advice given re development of high throughput screening methodology for identification of 3x plants
	Additional activities using supplementary funding 2014/15			
	Supply of Flow cytometer to VAFS	Instrument options reviewed and recommendation made. Purchase facilitated	March 2015	Transfer of payment to supplier facilitated by UTas.
	Training in use of new flow cytometer	Ms A Price from UTas to Hanoi to train Dr Chi's staff	April 2015	Ongoing contact to answer queries will continue post Project
	Operation funds for Mr Le Son Ph.D. program	Sufficient funds set aside for Mr Son to complete his JAF Scholarship research on molecular markers for Acacias	May 2015	Agreement with UTas Finance.
	Lab work for additional fibre samples for 3.9	Samples of rotation age A hybrid wood from Vietnam to	April 2015	Confirmation that fibres of 2 year old 4x equivalent to rotation age 2x. Data prepared for publication to be completed post-Project

		Hobart		
	Triploid discovery in OP seeds from 2x/4x hybrid trial (3.6.3)	Ploidy determination in Hobart from seedlots collected in Vietnam	May 2015	Low frequency of 3x seeds confirmed. Complex variation in ploidy within and between trees and pods within trees. Data prepared for publication post-Project. Ongoing investigations by Dr Chi and Mr Son.

7 Key results and discussion

Issues addressed on Objective by Objective basis.

Objective 1 – Development of new hybrid clones

The largest and most assured direct economic benefit from the Project will come from the deployment of a new generation of fully tested hybrid clones.

Confirming the performance of currently-available clones

The initial aim was to enhance utilisation of existing clones. Only about 10 hybrid clones are currently planted on a commercial scale in Vietnam, a small number considering the large size of the hybrid plantation estate. Performance of the clones in existing field trials was analysed and published. Clones BV10, 16, 32 and 33 developed in the north, and clones TB1, 6 and 11 developed in the south, are important commercial clones, and have been maintained as vigorous clones by repeated cycles of re-capture into tissue culture, with no decline in field performance of these clones evident over 2 decades (Kha et al 2012). These clones displayed similar growth performance in genetic trials at three locations in north, north-central and south Vietnam; some other more recently developed clones tested in the trials such as BV71-BV75 displayed similar good performance and are increasingly being planted on a commercial basis. The trials also confirmed the faster volume growth of selected hybrid clones compared to the two parent species in northern Vietnam, while in the south the best improved seed sources of *A. mangium* grew at a similar rate to the hybrid. Significant differences among the commercial hybrid clones for basic density (as indicated by pilodyn penetration) were established (Kha et al 2012).

This review did not lead to major changes in clonal deployment, because the first commercial clones developed in the early 1990s were found to still be among the fastest-growing clones, with acceptable wood density.

In the project as designed it was intended to screen existing and newly developed hybrid clones for wood properties, notably basic density and kraft pulp yield. Review of improvement objectives (Kha et al 2012) indicated that most growers in Vietnam, who sell their wood on the basis of green volume or green weight, would not benefit financially from increased basic density or pulp yield. However, increase in basic density and pulp yield is still desirable for long-term competitiveness of Vietnam's acacia plantation and processing industries. Other traits, including tolerance to diseases, wind and drought, are expected to become increasingly important, depending on the regions in Vietnam where clones are deployed, and the extent to which disease attack becomes a major problem, as it has in Indonesia and Malaysia (Nambiar and Harwood 2014).

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Developing a large population of new clones (short-term)

To generate a population of new hybrid clones we selected a large (5000+) population of putative hybrid seedling. These were identified in the nursery from seedling populations raised from carefully-chosen pure-species bulk and family seedlots collected at locations where adjacent seed orchards of the two species had been planted, to promote hybridization by open pollination. Putative hybrids were identified at frequencies of 1-3%. We then field tested these seedlings in screening trials, identified the most promising hybrid seedlings for cloning; and establish these clones in first stage multi-site trials in comparison with commercial clones as controls.

By 2010 a total of over 5000 putative hybrid seedlings together with controls were under test at 4 sites across Vietnam. From these plantings, 210 selections with superior early growth and stem form were made at age 2 years and cloned.

In a separate strategy implemented by IFTIB that was linked to the project, 340 putative hybrid selections were identified in IFTIB pure-species progeny trials and cloned at age 2

years. These two strategies thus yielded a total of 550 candidate clones which were established, together with commercial *A. hybrid* clones and pure-species seedlings as controls, in screening trials on 4 sites, in 2011-2012. Two-year assessments of the clone trial yielded several important results (Table 1, Figure 1):

- Some of the new clones displayed rapid early growth, equalling or exceeding the commercial hybrid controls
- Both *A. mangium* and *A. auriculiformis*, as female parents, yielded many promising new clones
- The Project method of two-stage selection (hybrid seedling trials with intensive selection of best hybrid seedlings for inclusion in clone trials) yielded faster average growth and a higher proportion of good-performing clones than did selection of putative hybrid candidates from the pure-species progeny trials

Table 1. Mean height (m) at ~ 2.5 years of age for different clone categories in four Project clone screening trials

Clone trial	Commercial controls	New clones from *A. auriculiformis* mothers		New clones from *A. mangium* mothers	
		Method 1*	Method 2**	Method 1*	Method 2**
Ba Vi (North)	9.6	-	6.5	8.5	8.5
Dong ha (Central)	8.9	-	5.5	-	7.3
Bau Bang (South)	6.3	6.6	4.4	6.7	5.5
Bac Giang (Northeast)	8.7	7.8	5.1	7.4	7.1

*Method 1 is Project method (testing putative hybrid seedlings in field trials, selecting best for clone testing at age 2 years)

**Method 2 is selecting all candidates with hybrid morphology in pure-species seedling progeny trials

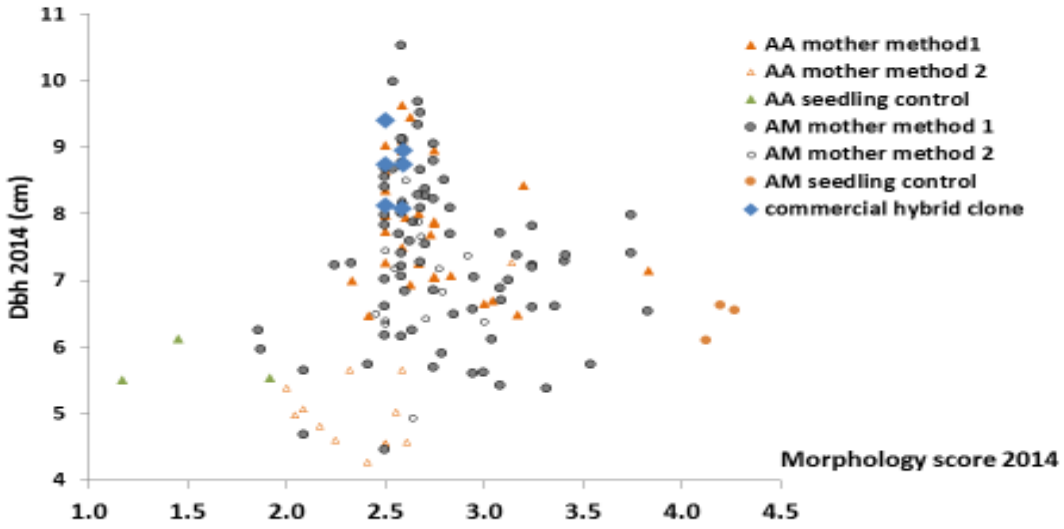


Figure 1. Diameter at breast height (Dbh) of individual candidate clones and controls at the Bac Giang clone trial in northeast Vietnam, plotted against morphology score (1 = morphology of pure-species *A. auriculiformis*, 5 = morphology of *A. mangium*)

Figure 1 shows the growth (mean diameter at breast height) of individual clones in the Bac Giang clone trial in Northeast Vietnam, at age 2.5 years.

A likely reason for the superior average performance of candidates selected by Method 1 (two-stage field testing) was the higher intensity of selection. The best candidate hybrid seedlings were selected for cloning and re-testing at a frequency of about 1 in 20, whereas all hybrid individuals in the pure-species trials (Method 2) were cloned for testing in the clone trials. Future selection of candidate hybrid genotypes in pure-species breeding trials would be more efficient if only the best hybrid candidates are selected and cloned for testing. Another possible contributing factor is that Method 1 focused on seedlots collected from stands of diverse but selected *A. mangium* and *A. auriculiformis*, deliberately planted adjacent to one another with a view to generate superior hybrid combinations (these paired stands were an output of the ACIAR-funded Domestication of Australian Trees Project, implemented in 1999-2004).

Morphology scoring conducted in the clone trial at this age (later checked by molecular marker assessment) suggested that some of the candidate clones were complex hybrids (e.g. backcrosses between one or other of the pure species as the maternal parent and acacia hybrid as the male parent), or pure *A. auriculiformis* genotypes, rather than F_1 hybrid combinations between the pure species (Figure 1). This raises important issues for both hybrid breeding and pure-species breeding. Identifying hybrid individuals on their morphology was particularly challenging when screening *A. auriculiformis* seedlots, and many seedlings identified as putative hybrids at the nursery stage were on later assessment at age 1-2 years considered to be pure *A. auriculiformis*. Molecular markers that can distinguish between genotypes of the two pure species, F_1 hybrids and back-crosses between the F_1 and either pure species, currently being developed by current UTas JAF Mr Le Son, will be extremely valuable in the context of hybrid identification.

A sub-set of the most promising clones identified in the clone screening trials are being selected for more comprehensive testing by IFTIB, including assessment of disease and

pest tolerance, stem and branch form, wood properties and stress (wind and drought) tolerance. This testing is scheduled to commence in 2016. Superior, well-tested new clones could, in principle, be tested and receive MARD approval for subsequent commercial deployment by 2020 or soon thereafter, substantially expanding the genetic base of clones available to Vietnam's growers.

Advanced breeding of new hybrid clones (longer term)

In order to produce a new generation of hybrid genotypes under more controlled conditions a new grafted hybridizing seed orchard incorporating the 10 best selections from each of the VAFS *A. mangium* and *A. auriculiformis* pure-species breeding populations was planted at the IFTIB research station at Phu Tan in southern Vietnam. This location was chosen because flowering was expected to be good, and some degree of reproductive isolation of the orchard from other populations of the two parent species can be achieved. The orchard was planted in 2013, and the first flowering was in 2014, first seed collections were made in April 2015, and it is expected that a good seed harvest following good general flowering of the entire orchard can be made in 2016.

Another route to production of new hybrid genotypes is controlled pollination between selected pure-species individuals. Because this is labour-intensive, only a few combinations can be produced each year. However, over 3700 diploid hybrid seeds from combinations among 10 superior parents were produced as control treatments in the course of Dr Chi's PhD studies (Nghiem et al. 2013). Over 1300 of these seeds were successfully germinated and in 2014 were established, together with commercial hybrid clones as controls, in a field screening trial at Ba Vi. The best individuals will be identified at age 2 years for clonal capture and testing, as per Method 1 for the hybrid seedlings described above.

A third long-term method for hybrid breeding was commenced in 2014 by selectively thinning the clone trial at Bau Bang. The best 60 clones in this trial were retained and the stand opened out to encourage production of advanced-generation hybrid seed, which will be collected, germinated and screened in field trials to identify favourable genotypes. F_2 and subsequent generations of advanced hybrid outcrosses will generate, through recombination, genotypes that can never be produced in the F_1 generation. Eventually, it may be possible to produce a stable advanced-generation hybrid strain that can be propagated by seed.

The different routes to the breeding of new hybrid genotypes are summarised in Table 2.

Table 2. Different methods of hybrid breeding for generating new acacia hybrids

Method of hybrid breeding	Comments
Identification of putative hybrid individuals at the nursery stage in open-pollinated seedlots collected from adjacent stands of selected *A. mangium* and *A. auriculiformis*	Proven in this project. Two-stage screening (trials of putative hybrid seedlings identified at the nursery stage followed by capture of the best individuals and clone screening trials) appears most efficient approach. Difficult to distinguish hybrid genotypes at the nursery stage in seedlots raised from *A. auriculiformis* maternal parents.
Identification of putative hybrid individuals in young pure-species progeny trials	Tested in this project. Yield of superior hybrid genotypes will depend on whether pure-species seedlots are derived from parent trees exposed to appropriate pollen sources, and the selection intensity applied when selecting candidates
Dedicated clonal hybridizing orchard of elite genotypes of *A. mangium* and *A. auriculiformis*	Developed in this project. If the orchard is reproductively isolated to minimize contamination from external pollen, male pedigrees of open-pollinated seed can be recovered using molecular markers. Can also be used for production of hybrids by controlled pollination.

Controlled-pollination between selected *A. auriculiformis* and *A. mangium* trees	High-cost, high requirement of skilled labour, but full pedigree is retained. Crosses can be made between parents superior in desired traits.
Open-pollination among hybrid clones to develop advanced-generation hybrid breeding population advanced by recurrent selection	Potential to eventually develop a stable hybrid line for seed-based deployment, although may also yield superior individual clones in the short term. Recombination yields advanced-generation hybrid genotypes with genomic combinations that cannot be obtained in the F_1 hybrid. Yet to be proven for acacias.
Polyploid hybrid breeding	Discussed under Objective 3. Potential to develop sterile clones with qualitatively different wood properties.

A comprehensive hybrid breeding strategy, incorporating all these approaches, was documented and presented as a poster at the Acacia 2014 conference, and discussed in more detail by (Harwood et al. 2015). All approaches have some common elements and challenges:

- deployment will be clonal, requiring clonal testing across contrasting climatic regions in Vietnam and ranking of clones in each environment
- improvement objective (the relative weighting given to different traits including growth, stem and branch form, tolerance to diseases, pests, wind and drought, wood properties) remain unspecified.

Improvement objectives may change over time and will vary across different regions of Vietnam with different climatic and disease challenges, and for different grower categories such as pulpwood and sawlog growers. It is therefore desirable to maintain broad-based pure-species breeding populations to support hybrid development.

Thorough clone testing of candidate hybrid genotypes is essential. This involves initial clone screening trials testing hundreds of candidate clones in designs that use small treatment plots (as has been done in this Project) followed by second-stage clone proving trials in which promising clones are tested in the target planting environments with trial designs using large block plots, to accurately determine their growth performance, form, wind tolerance and other traits, prior to final selection and deployment. Tests to screen for tolerance to major disease threats such as *Ceratocystis* are currently under development (Brawner et al. 2015). As testing information accumulates and grower priorities become clearer, deployment of individual clones will likely be region-specific and may also be targeted to particular categories of growers.

The finding that second-generation “pure-species” progeny trials of *A. auriculiformis* and *A. mangium* yielded many hybrid individuals, and that a proportion of these appeared to be back-crosses arising from A. hybrid pollen contaminating the first-generation breeding trials, carries strategically important implications for overall acacia breeding strategy in Vietnam. It will be difficult to maintain genetic separation and species purity of pure-species breeding populations, because *A. hybrid* is widely planted across Vietnam, and is in close proximity to most *A. mangium* and *A. auriculiformis* breeding trials.

In order to secure tracking of pedigrees in the increasingly complex breeding programs for both pure species and hybrids, and indeed other taxa being bred by VAFS, we set up a genetic information management system. After investigating various alternatives, a simple information management system was developed using Microsoft Excel software, which is familiar to Project partners. At Project completion, the system had been fully developed for IFTIB’s pure-species breeding populations *A. auriculiformis*, *A. mangium* and *A. crassicarpa*, and was being extended to cover all acacia hybrid clones and polyploid breeding populations of acacia hybrid and pure species. The system is described in Appendix 1.

Objective 2 – To refine and demonstrate new deployment strategies for sexually propagated *A. mangium*

The system developed by the Project has been fully documented (Warburton et al. 2014, http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/Objective_2/Warburton_Evaluating_Clonal_Family_Forestry_as_a_deployment_strategy_for_Acacia_mangium_in_Vietnam__FINAL_2014.pdf) It is shown in Figure 3. Seedlings were transplanted into sand beds which were irrigated and fertilized with liquid fertilizer solution. Repeated harvests of shoots were collected and the number of plantable cuttings determined for each harvest. Trials established the percentage rooting obtained from the cuttings at different times of the year. This allowed calculation of the mean number of rooted cuttings produced from each planted hedge plant on a seasonal and yearly basis.

Figure 3. Experimental set-up for CFF at Cam Quay nursery, Ba Vi.

The first experiment in 2010 ranked three liquid fertilizers, available in Vietnam, and identified the solution (Fertilizer 3) giving the highest yield of cuttings (Figure 4).

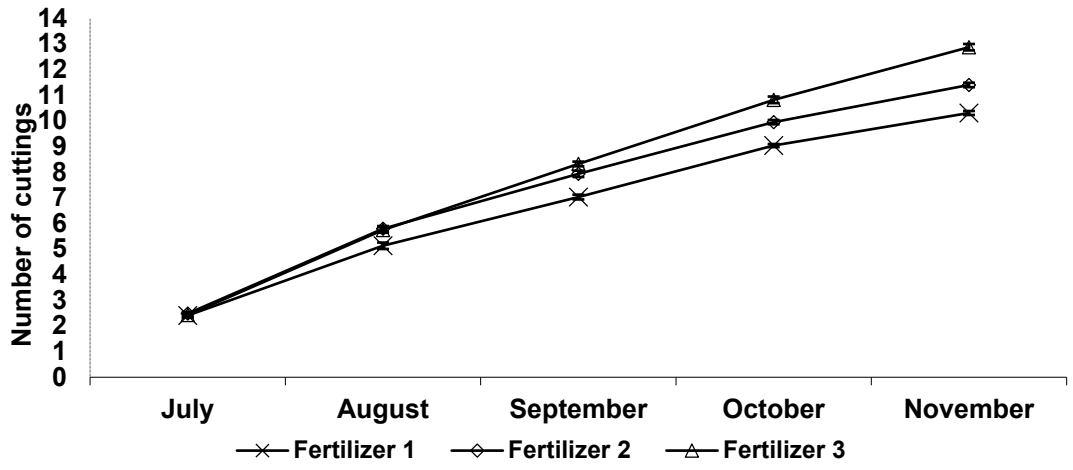


Figure 4. Cumulative cutting production in the first CFF experiment at Ba Vi

The rate of cutting production declined after November. 80-90% of cuttings were successfully rooted in July and September, but only 45-75% of cuttings rooted in November (late autumn), setting a limit to the season for raising cuttings.

Multiplication rates for CFF with *A. mangium* and *A. crassicarpa* in the highly seasonal climate of northern Vietnam were insufficient to warrant uptake of CFF there.

High rates of cutting production and rooting of harvested cuttings (generally over 80%) were achieved during the summer months, but both declined in the autumn and cutting production was not possible in the winter months under the prevailing conditions of low temperatures and light intensity.

Overall, the multiplication rate was in the range 9-14 cuttings produced per hedge plant, depending on the timing of hedge plant establishment and harvesting schedule (Table 3).

Table 3. Hedge plant and bed cutting productivities achieved, Experiment 3, Ba Vi

	Bed			
	1	2	3	4
Establishment date	April 2011	October 2011	October 2011	April 2011
Harvest schedule	Monthly	Weekly	Monthly	Weekly
Mean number of harvested hedges (n=720)	451	323	477	243
Cumulative number of harvested shoots (2012)	7211	9814	6963	9143
Cumulative number of rooted cuttings (2012)	7146	8235	6435	7425
Number of hedges established	720	720	720	720
Mean cumulative number of rooted cuttings per hedge planted	10.0	12.2	9.2	11.1
Rooted cuttings per m^2 in 2012 season	496	572	447	516
Cumulative number of rooted cuttings (2011)	1378	0	0	1657
Mean cumulative number of rooted cuttings per hedge planted (2011)	2.3	0	0	2.7
Total yield per planted hedge (2011+2012)	12.3	12.2	9.2	13.8

Weekly harvest of cuttings from hedge plants established in spring (April 2011) and maintained over two growing seasons, gave the highest multiplication rate of 13.8 plantable cuttings per hedge plant (Table 3). However, a large proportion of these cuttings could not be used in practice, because by the time cuttings were rooted and raised to planting size, which requires 10-12 weeks, the time window for field planting in northern Vietnam had passed (Figure 5). South and central Vietnam have more suitable climates for CFF, with better matching of cuttings production to the field planting window (Figure 5), and less constraint to cutting production in the winter months but the technology is yet to be fully tested in these regions. It may be noted that *A. mangium* is planted primarily in northern and north-central Vietnam, where prospects for CFF are least favourable. CFF may prove to be an appropriate technique for *A. crassicarpa*, another species not amenable to clonal forestry which is most prospective for planting in central Vietnam.

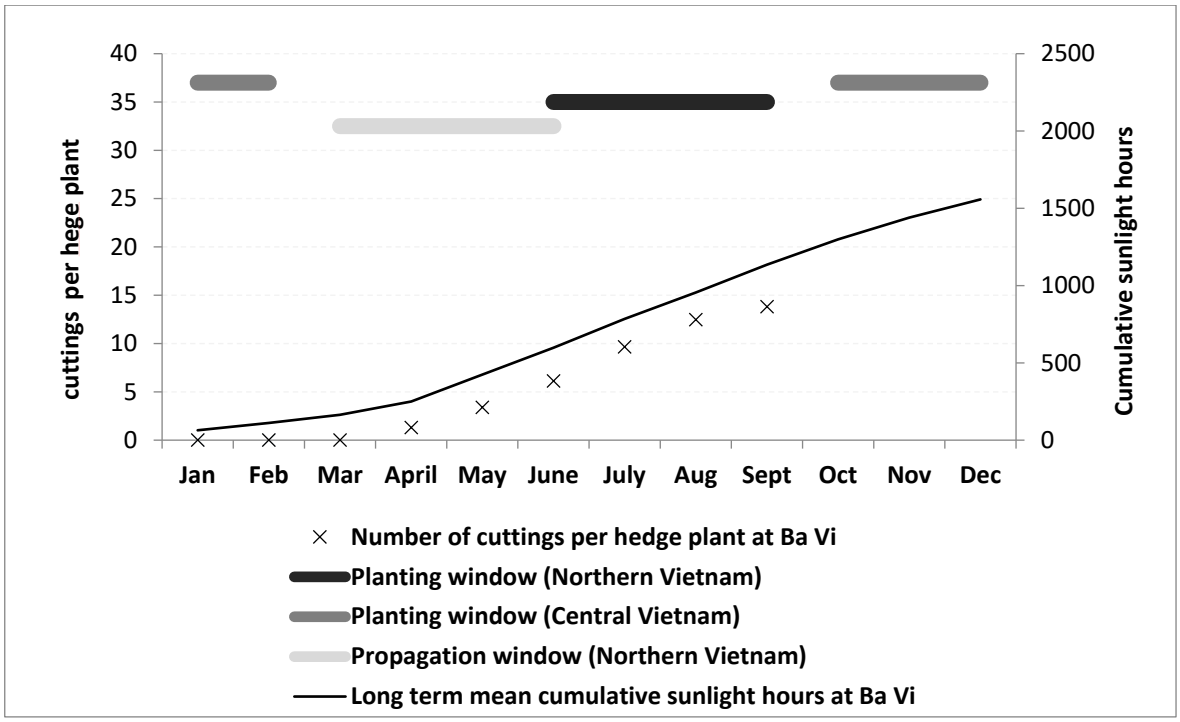


Figure 5. Cutting production at Ba Vi in relation to field planting windows in northern and Central Vietnam, and cumulative sunlight hours at Ba Vi

A field trial indicates that growth and survival of CFF cuttings was identical to that of seedlings of the ten *A. mangium* families used in the CFF experiments (Table 4). This was an encouraging result, because it shows that in northern Vietnam, *A. mangium* hedge plants can be over-wintered after their first season of cutting production, followed by continued cutting production in the second season, without any decline in the quality of planting stock raised from cuttings.

Table 4. 2-year growth of *Acacia mangium* CFF cuttings compared with seedlings of the same 10 families at Ba Vi, Northern Vietnam

Planting stock type	Height (m)	Diameter at breast height (cm)	Survival (%)
Seedlings	5.8	6.1	96
Cuttings from older (12+ month) hedges	5.8	6.1	95
Cuttings from young (4+ month) hedges	5.9	5.9	96

The major activity in this component of the Project did not deliver the projected benefit in terms of operational uptake of Clonal Family Forestry. IFTIB is addressing other strategies to mass-multiply the best, tested seed families of *A. mangium* and *A. crassicarpa* to expand their availability for operational planting.

Objective 3: Continue development and evaluation of promising new polyploid varieties of *A. mangium* and its hybrid

The polyploid hybridising orchard at Bau Bang, containing alternating rows of 4x *A mangium*, 2x *A mangium* and 2x *A.auriculiformis* (Nghiem et al 2011) established as an FST2003-002 activity, was a core resource for the Project work program. It began to produce seed in 2006 and was used for the breeding system study of Mr TD Vuong (Vuong 2010, Griffin et al 2012) and for investigations of reproductive biology of the cytotypes (Nghiem et al 2011, 2013). The knowledge from those studies helped underpin the polyploid breeding strategy development during the Project but they were funded by the John Allwright Fellowship and were not formally part of the Project work program. The papers may be consulted for the results which are not repeated in this Report.

Characteristics of 4x A.mangium trees in contrast to 2x

The tetraploid trees differed from normal diploids in many respects (Harbard et al 2012). Gigantism is expressed as larger, thicker, cup shaped phyllodes, larger stomata at lower density and thicker more tessellated bark (Fig. 6). Polyads and flowers are also larger (Nghiem et al 2011).

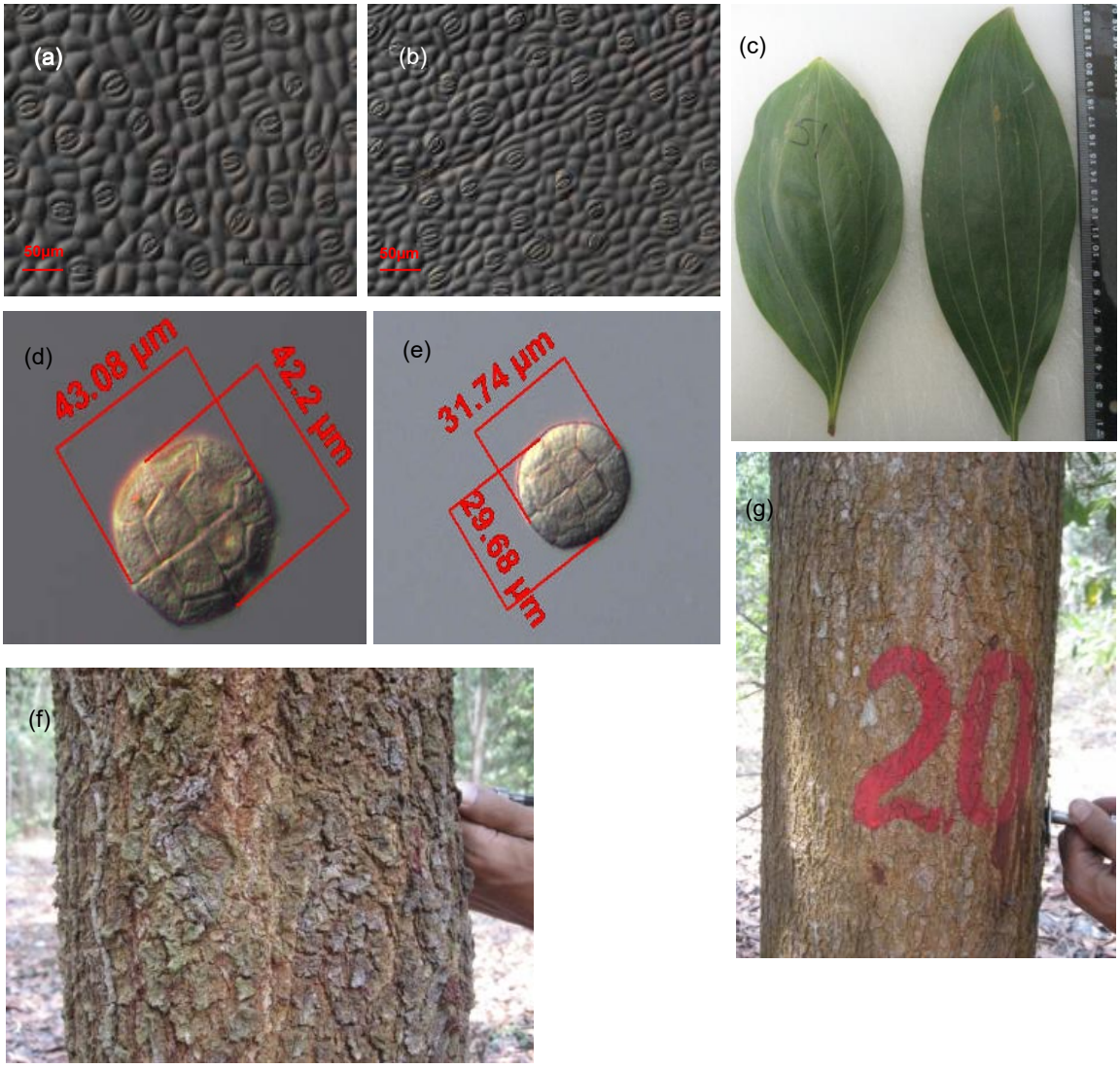


Figure 6 Gigantism characteristics of polyploid *A. mangium. Mean length of 4x stomates*

are 24.3µm with mean density of 23 / FOV (a) compared to 2x mean length of 20.9 µm and density of 36 / FOV (b). The 4x phyllode on the left in (c) is thicker and wider than the 2x phyllode on the right with typical cup shape compared to the flattened 2x. 4x polyads (d) are 20% larger than 2x polyads (e). Thicker, tessellated bark of the 4x stem (f) is in contrast to the smoother 2x bark (g)

The phyllode characteristics affect water relations of the plant (O'Grady et al unpubl data) with the 4x dessicating at a slower rate than 2x. This could be of interest in terms of drought tolerance though we are yet to demonstrate this at the whole plant level in the field. The bark of 4x trees is thicker with more cork cambia leading to a flakier or tessellated morphology (Drinnan unpubl data) and we investigated whether this was associated with any higher levels of phenolic extracts which might be of interest to industry producing tannin or other related chemical products. The methodology for assaying this proved challenging but a reliable protocol was developed (Technical Report Harbard et al (2014) http://www.breedingtropicalacacias.com/page20.php. As this was not part of the formal Project work plan we were unable to progress the study beyond obtaining initial indications that bark from 4x OP progeny of family 11 contained significantly higher levels than both 4x family 22 and 2x samples. Variation between trees indicates that selection on phenol content would be a possibility. Further work would depend on interest from industry...we know there are buyers of acacia bark in Vietnam but the end use is unclear.

The major economic benefit of gigantism in trees derives from an increase in fibre dimensions, and the associated change in properties of pulp and paper made from the wood and/or from the effect on strength of manufactured solid wood products. Study of these attributes on 8 year old acacia wood from the Bau Bang hybridising orchard was a major Project activity. Trees of both 4x and 2x *A.mangium* were felled and chips prepared and forwarded to the SAPPI laboratory in South Africa. Full results were published in Griffin et al 2014. Compared with 2x the 4x trees were found to have longer, wider, stronger fibres; a similar Pulp Yield% but less chemical use to constant kappa; and Kraft pulp with higher bulk, porosity and tear strength making for similarity in these traits to softwood pulp.

The following figure from that paper illustrates the cytotype differences in fibre dimensions.

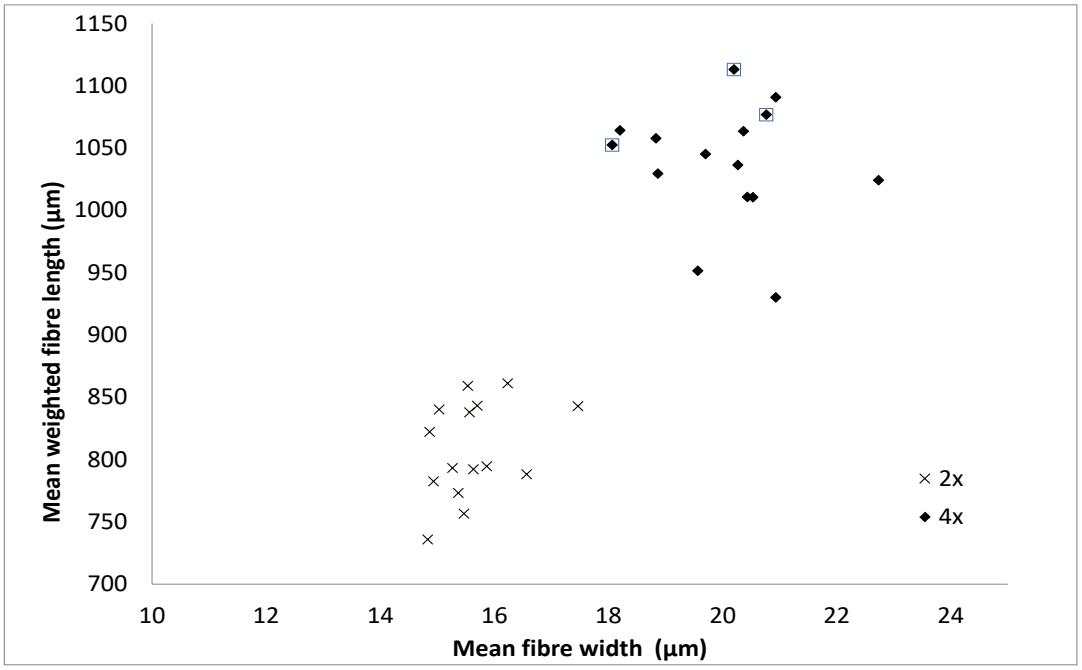


Figure 7 (from Griffin et al 2014) Relationship between fibre length and width for macerated chip samples

We have also produced as yet unpublished data that demonstrates the increase in fibre dimensions is heritable. The fibres of 2 year old 4x OP progeny of 2 of the original Shell Clones are longer than 2x of equivalent age (fig.8), and in fact about the same length as in rotation age acacia trees

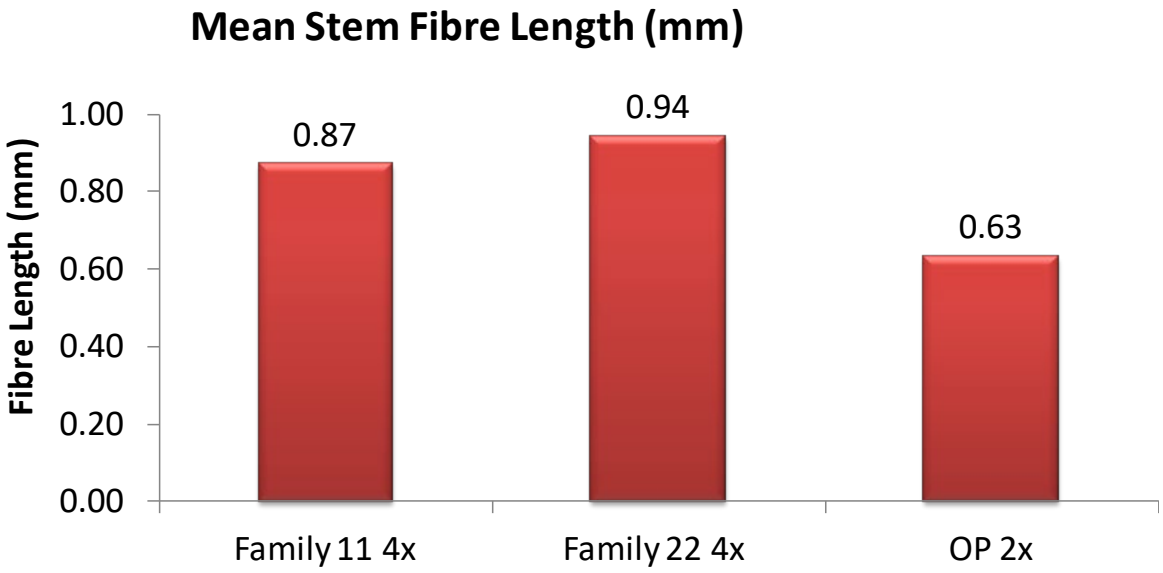
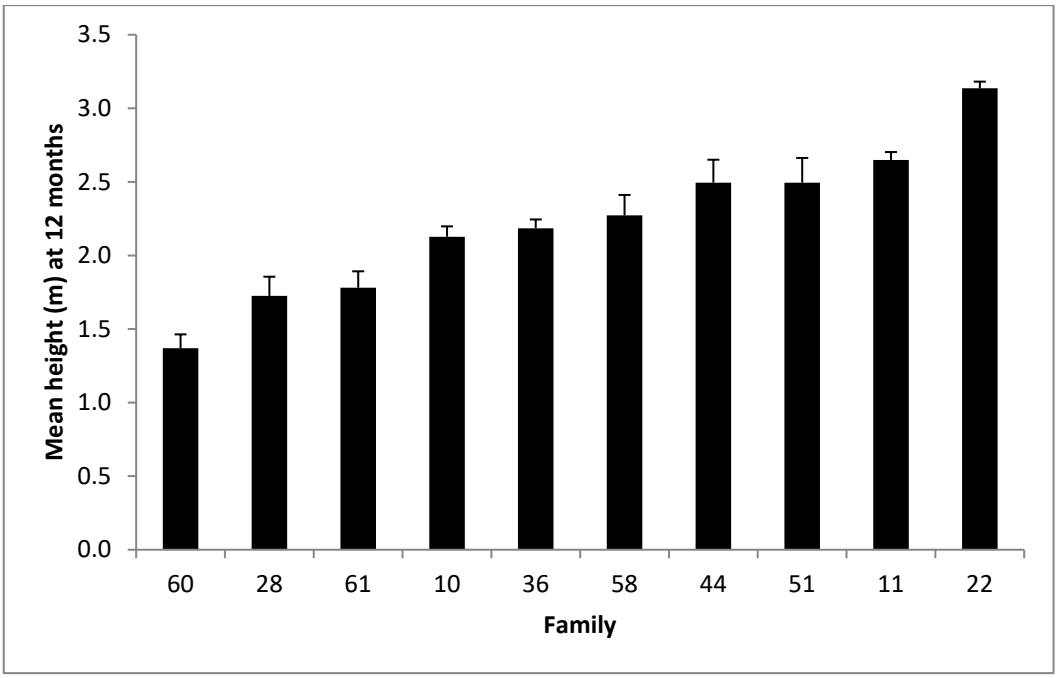


Figure 8. Mean fibre length (mm) from disks taken from 2 year old trees in the Bau Bang OP Progeny trial and from an adjacent commercial control. (Harbard unpubl. Data)

Growth of Open Pollinated 4x Progeny:

The studies of Mr TD Vuong (Vuong, 2010) showed that chromosome doubling changed the breeding system of the 4x Shell Clones from outcrossing to predominantly selfing. Such a change has been noted in many plant taxa and the possible reasons are discussed in Griffin et al (2012). Since most outcrossing species including these tropical acacias exhibit inbreeding depression (reduced vigour and higher frequency of aberrant seedlings) this presented a serious and unplanned impediment to our initial strategy of producing 4x seed by open pollination on an operational scale. We planted a trial of open pollinated seedlings from 10 of the Shell Clones in Bau Bang Orchard, with results summarised in the following figure from Griffin et al (2015).



Family	60	28	61	10	36	58	44	51	11	22
Survival %	37.9	51.1	75.0	77.4	70.6	40.0	70.4	24.7	81.3	84.6
Ht CV %	39.5	37.2	28.5	23.1	24.4	28.5	27.3	30.0	24.5	17.8

Figure 9: Early height growth and survival of open pollinated progenies from 4x mothers in Bau Bang hybridizing orchard. Standard errors of family means are shown (from Griffin et al 2015)

Families 11 and 22 showed the best and most uniform growth performance with many good quality trees with straight single stems (unlike most 2x trees which are now planted) and it was considered worthwhile to replant these in a designed experiment to calibrate growth against 2x planting stock. This trial was planted at Ba Vi in northern Vietnam. After 11 months the 2x controls averaged 3.7m in height while the 2 4x families were similar to each other and averaged only 2m. Clearly it is not possible to recommend planting of such seed, so the focus must be on retaining the wood property and form attributes demonstrated by the 4x while increasing their vigour by breeding outcrossed advanced generation varieties…or perhaps concentrating solely on production of 3x hybrid clones for deployment (see below).
The best of the trees in the blocks of family 11 and 22 have been grafted into the Phu Tan farm arboretum and can be used for study of S1 generation breeding system and for advanced generation breeding of 4x and/or production of triploids by intercytotype crossing.

Production and Verification Protocols for Colchicine Induced Tetraploids:
The original Shell Clones were produced by colchicine application to seeds of *A.mangium.* Since we wished to produce a relatively large number of new stable 4x lines it was necessary to do our own optimisation experiments. Successful conversion to a stable

tetraploid requires management of the balance between effective level of colchicine and duration of exposure that will double the genome of each cell in a targeted meristem and not adversely affect survival. The protocol which worked best for us was to place the seeds on filter paper soaked with 0.02% colchicine for 24 hours at 29 C. The same protocol also worked well for *A.auriculiformis* and *A.crassicarpa.* On average we were able to produce 10 stable 4x plants per 100 seeds treated (Harbard et al 2012, Lam et al 2014, Harbard et al 2014)

Across all of our polyploid induction experiments half of the surviving plants were mixoploid. As noted it is essential to assay the seedlings at least twice with 2 phyllodes taken per plant at each sampling to demonstrate stability and then to repeat the assays on propagules when they have been multiplied for field planting. Flow cytometry was found to be the most accurate and cost effective method for identifying ploidy. Visual identification has been shown to be prone to error as colchicine can alter plant morphology without affecting ploidy. Chromosome counts on roots is time consuming and made difficult by their small size. Size and density of stomata is relatively easy to assess but will only identify the ploidy of the epidermal cell layer which originates from a single layer of the meristem and may be of a different ploidy to the cells beneath. In contrast, flow cytometry assesses all cell layers in the phyllode and has a high throughput with between 40 and 60 samples measured per day. For protocols see Harbard et al (2012), Lam et al (2014)

We also induced 4x lines from tissue cultured shoots of 4 commercial hybrid clones in order to see if this would give a fast track route to production of deployable polyploid clones. Optimisation experiments showed that the best treatment was immersion in 0.1% colchicine for 48hr, at 29 C and 100rpm. Extended shoots were screened for ploidy and those which showed 4x were recultured. Once again repeated flow cytometer assessments were required before a culture of apparently stable 4x shoots could be sent back to Vietnam for further multiplication to numbers required for field trialling Price et al (2011)
http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/Objective_3/Price_Acacia_hybrid_micropropagation_tetraploid_induction_May_2011.pdf .

Establishment of New 4x Seedling Lines in Vietnam:

A total of 58 new seed based *A.mangium* lines were developed in Hobart and transferred as unrooted cuttings to Vietnam where they were multiplied and planted in blocks at Phu Tan research farm. A recent inspection indicated that the trees are growing well and that they may begin to flower in 2015/16. The main aim of this material is to find clones which are comparable or better than the best parents from the Shell Clones (11 and 22) and to use this in advanced generation 4x breeding and also as parents in making new 2x x 4x crosses for production of 3x trees for evaluation as clones. As soon as a good seed crop is produced the work program followed at the original Bau Bang orchard

The same procedure was followed in exporting a population of 70 4x clones of *A.auriculiformis* and 48 clones of *A.crassicarpa* with the successfully captured clones established at Phu Tan. Within 3 years these will provide VAFS with the opportunity to derive new 4x hybrid clones by inter-specific crossing. Attempts to make crosses with *A.crassicarpa* at the 2x level have proved unsuccessful but there are examples in crop breeding where hybrids are more easily made at the 4x level. If this proves true then combination of the complementary adaptive traits of these species could greatly increase productivity on difficult planting sites. It is worth noting that clonal forestry in eucalyptus is largely with vigorous well adapted and disease resistant hybrids ...in acacia we only have the one combination of *A.mangium x auriculiformis.*

Establishment of 4x lines of Commercial Hybrid Clones in Vietnam:

As noted clones were returned to Vietnam and multiplied together with 2x versions of the same genotypes. These were planted in a replicated trial at Bau Bang so that we could assess the differences in the cytotypes in a common environment. The 4x ramets of all the clones had broadly the same morphology..stronger apical dominance as observed in the S1 4x progeny, and larger tougher phyllodes (fig. 11). We did not measure leaf areas but it appears that the 2x plants carried a higher leaf area at this early growth stage. Since growth is dependent upon photosynthate produced by the canopy the faster growth of the 2x cytotypes was not unexpected. The differences were not as great as in the seedling trial described above, and there were some differences between clones but still the growth was clearly inferior and utility of this generation of polyploid hybrids would depend on demonstration of adaptive benefits which affect volume yield per ha. other than through growth rate of individual trees. One such example might be decreased susceptibility to wind damage associated with the smaller lighter crowns. This possibility needs to be tested by planting in high risk areas.

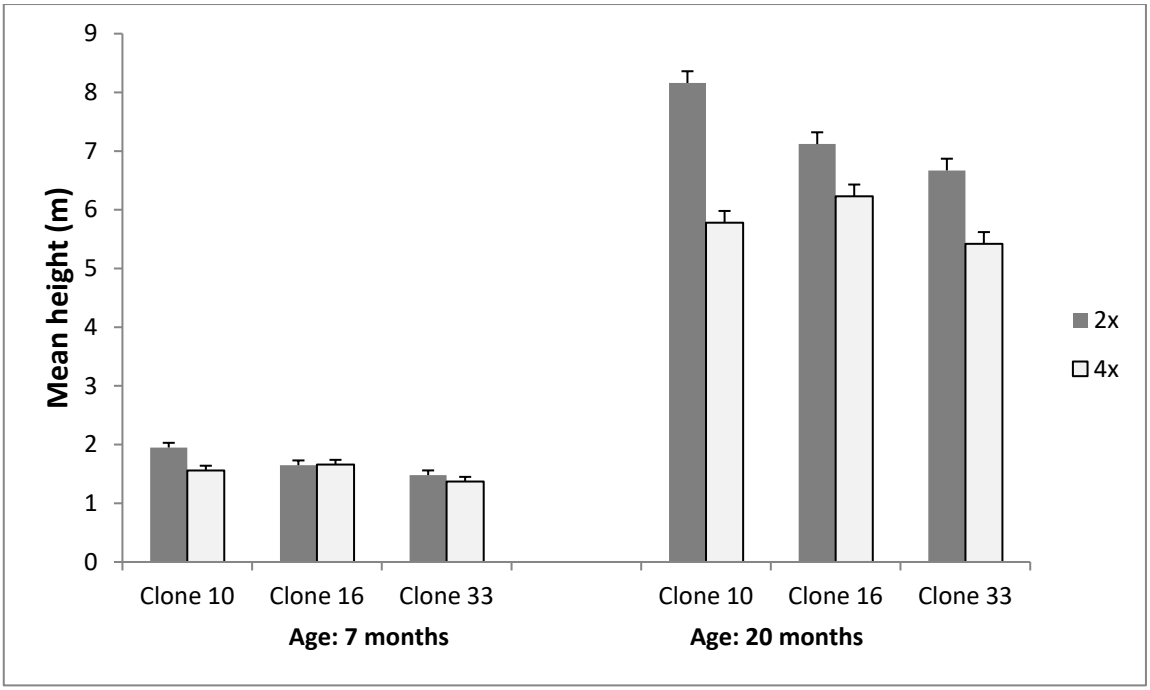


Figure 10 (Griffin et al 2015) Early height growth of colchicine induced 4x lines derived *in vitro* from three commercial hybrid clones and their respective diploid cytotypes. Differences between clones, cytotypes and clone*ploidy interaction term are all significant at P<0.001 Error bars show standard error of difference of treatment means at 7 and 20 months.

Figure 11: Ramets of 2x hybrid clone BV33 (L) and its derived 4x line 33-667 (R) 11 months after planting in a replicated trial at Bau Bang, Vietnam. Note stronger apical dominance and fewer larger phyllodes on the 4x. Cytotype differences in morphology substantially consistent across clones (from Griffin et al 2015)

Production and testing of 3x Genotypes

There are two approaches to the production of triploids: via inter-cytotype crosses which can be either inter- or intra-specific and by open or controlled pollination, or through discovery of 3x progeny of diploid mothers produced by open pollinated matings involving unreduced gametes from 2x trees (that is gametes which have a diploid rather than haploid chromosome complement). Both male and female unreduced gametes are produced in many plants at a low frequency as a result of errors in meiosis (Bretagnolle and Thompson 1995). During the course of the Project and, through VAFS efforts in the extension year, we selected and propagated a total of 16 3x clones produced by both methods and field evaluation trials commenced in 2014. It is already evident that the triploids (which are by definition sexually produced outcrosses) are faster growing than 4x trees and comparable to the commercial hybrid clones.

The outcome of inter-cytotype crossing with 4x *Am* lines and both *Am* and *Aa* diploids was described by Nghiem et al. (2013). Although she identified no strong pre-zygotic barriers to mating, no mature pods were obtained when the 4x was used as a mother. The crosses of 4x *Am* pollen to diploid mothers of *Am* and *Aa* yielded a few pods (1.03% of flowers pollinated) compared with 7% set in diploid – diploid controls. There were also fewer filled seeds per pod (0.8 and 0.3 for each species compared with 9.4 and 5.5 respectively). Although some developed 3x seeds were obtained they generally failed to germinate. Subsequent work has shown that it is possible to rescue such seeds using tissue culture (Nghiem et al. 2014). Thus we do have a practical although rather laborious way of producing a population of 3x plants which can be cloned for field evaluation. VAFS is continuing with such crossing programs and has so far produced 2 clones which are showing excellent early growth in a field trial.

During the Project we used flow cytometry to evaluate the ploidy of open pollinated progeny from the hybridizing orchard. Initial focus was on seedlings from 4x mothers but

when we had screened 214 from 13 clones without identifying any triploids we switched focus to 2x mothers. 1 of 268 seedlings from *Am* and 2 of 274 of *Aa* were 3x (Harbard and Nghiem 2014). The latter exhibited no hybrid morphological traits so we can be confident that they derived from matings of a haploid and an unreduced gamete of diploid Aa trees. The *Am* triploid could possibly be derived from mating with pollen from a 4x tree. However we have other evidence that *Am* can produce 3x progeny in a purely diploid population. In the course of colchicine induction experiments with seed of all three species from orchards which were solely 2x we screened over 1000 seedlings and found 3x seedlings at an overall frequency of 1 in 170 (Harbard et al. 2012, Harbard unpubl., Lam et al. 2014) which agrees well with the result from the hybridizing orchard and is similar to that found in seedlings from two natural populations of *A.dealbata* (Blakesley et al. 2002). During the extension year UTas and VAFS staff worked on ways of improving throughput on the flow cytometer using a two stage sampling process. It is recommended that VAFS apply this method to future screening of progeny from the elite hybridising orchard in addition to making and screening inter-cytotype crosses.

As reported above, no triploids were identified in our initial screening of open pollinated seed from 4x mothers in the hybridizing orchard (Griffin et al. 2012). However in the field trial of OP progeny from the orchard the most vigorous individual in a block of 214 seedling progeny of clone 22 was found to be triploid. The most likely explanation is that this originated from pollination with a normal haploid polyad from one of the adjacent diploid trees. The tree has now been cloned so that we can evaluate its field performance and for studies of the reproductive biology.

Reproductive Biology of Triploids:

One of the key objectives was to produce acacia clones which are effectively sterile, so reducing concerns about weediness and invasion of native floras which has occurred with other species of acacia in other ecosystems (Richardson et al 2015)

Since it took a long time to find/produce 3x clones and then we had to wait until they matured sufficiently to flower, we were unable to complete proof of concept studies within the term of the Project. However we obtained some initial data and VAFS have followed up to the point that we are in a position to publish during 2015/16.
Grafted ramets of two of the 3x *Am* clones flowered for the first time in 2013 and observations were reported by Harbard and Nghiem (2014). Inflorescence production was similar to 2x trees but no open pollinated pods were set. 86% of polyads appeared abnormal and no germination was observed either *in vitro* or *in vivo.* Diploid pollen applied to stigmas of 3x flowers grew normally into the ovary but there was no resulting pod set. Similar results were obtained from the follow up studies by VAFS in 2015 and it is reasonable we can conclude that prospects are good for achieving our objective of producing clones with zero or greatly reduced potential for weediness.

Conditions for Commercialisation

We have shown that polyploid trees have improved fibre and pulp properties of potential interest to pulp and paper makers and higher stiffness which adds value to solid wood products requiring strength properties. The good form of the trees should increase merchantable volume but there may be a cost in terms of volume MAI and as long as growers are paid only for volume production they would have no incentive to plant. A study conducted with the advice of Dr C Clarke, a wood supply expert from SAPPI in South Africa, (Griffin et al 2013) Unpubl. Project Discussion Paper **"**Towards commercial deployment of polyploid Acacia in Vietnam"
http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Griffin_et_al_Commercialisation_Study_Report_August_2013.pdf considered that for a 100,000T pulp mill a minimum of 4500ha of tetraploid wood would make a useful

contribution to mill feedstock and to associated solid wood production. So who would take the risk of planting this wood in the expectation that they would receive an adequate return at harvest? Clearly a vertically integrated company producing wood for own use would be best placed but none of those exist in Vietnam and the export commodity market is a risky proposition. We offer no solution to this market development problem, but note that it needs to be faced by VAFS once they have full confidence that the new varieties are commercial ready.
As noted above the current focus is on producing 3x rather than 4x clones for operational use and when the wood properties of those (faster growing) clones is determined the commercialisation options review could usefully be repeated.

Outline of Advanced Generation Breeding Strategies

Practical improvement strategies need to take account of the biological realities of the target variety. In the case of these tropical acacias key traits are the self fertility of 4x and effective sterility of 3x; the extreme difficulty of producing breeding seed by controlled pollination; and the fact that both *A.hybrid* and *A.auriculiformis* can be cloned on a commercial scale but that this is much more difficult for pure *A.mangium*. With this knowledge in mind we made a comprehensive review of the literature on polyploid breeding and gene expression and also of the general benefits of high heterozygosity in trees and other outcrossing taxa, Strategies for improving 4x varieties and production of 3x trees for clonal evaluation were then developed for the next decade or more and presented in diagrammatic form in Griffin et al (2015). Figures 4a and 4b from that paper are reproduced here.

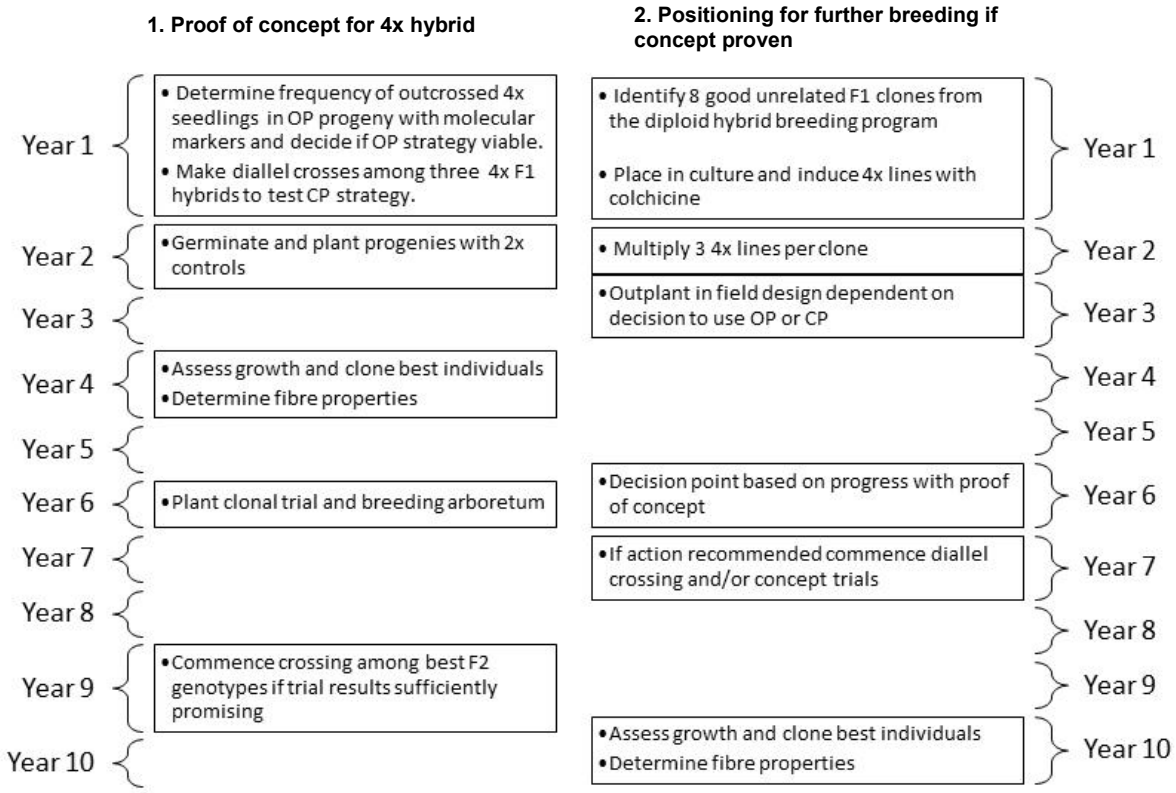


Figure 4a: Timelines for evaluation and development of strategy options for tetraploid breeding (status of germplasm in 2014 = Year 1)

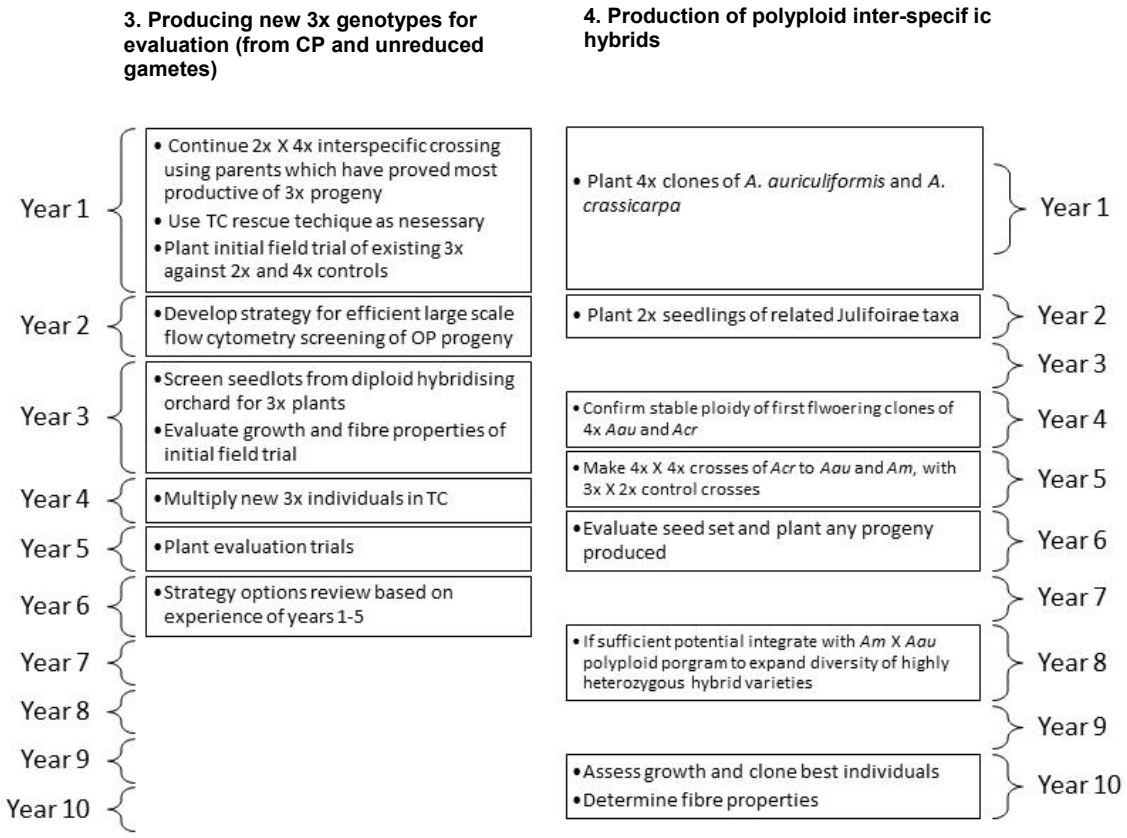


Figure 4b: Timelines for evaluation and development of strategy options for triploid and polyploid hybrid breeding (status of germplasm in 2014 = Year 1)

We conclude with the following quotation from Griffin et al (2015) as an encouragement to future breeders to persist with the program for all the reasons espoused in that paper

"Dewey (1980) noted that one of the conditions of adopting a polyploid breeding approach was that the breeder had to be "prepared to invest a professional lifetime to the project ". This sounds frightening until we note that acacia breeders have already spent over a professional lifetime picking the low hanging fruit of provenance variation and have subsequently made only modest gains from conventional recurrent selection programs"

It is worth being adventurous as we have only just begun the process of domestication of tropical acacias and are certainly nowhere near realizing their ultimate genetic potential.

8 Impacts

Text from the original Project document is presented below in italics, with each section annotated describing actual performance during the 5 year course of the Project and with comment on expectations for the next 5 years

8.1 Scientific impacts – now and in 5 years

This Project is expected to provide scientific impacts, through uptake of information provided in peer-reviewed publications in international journals, in at least four areas:

- *Optimized strategies for hybrid breeding, testing, selection and large-scale deployment of tropical acacias.* Two peer reviewed journal papers and two posters were published, and a journal paper reporting key results scheduled for 2016.
- *Improved acacia seed production management including new understanding of pertinent reproductive biology.* Through the work of our JAF scholars (Mr Vuong and Dr Chi) the Project has generated much new information about the reproductive biology of *Acacia* and in particular the polyploid varieties. This will be of lasting benefit to all scientists working on improvement programs of tropical acacias. The Project publication list includes 5 papers on this topic with up to 6 more planned before the Project terminates (see Section 9.3).
- *New applications of family forestry in operational deployment of acacia varieties which are difficult to clone.* Because the methodology was not judged to be economic in the test environment of northern Vietnam, no journal publication was produced. A comprehensive Technical Report was published on line (Warburton P. 2014) and a poster was presented at Acacia 2014 (Warburton et al 2014). Now that the technology is established in Vietnam there is a platform for application to other acacia taxa e.g. *A. crassicarpa* in regions of more favourable climate than the test location at Ba Vi. Project focus on mass-deploying best *A. mangium* families has stimulated VAFS to evaluate other options for CFF: multiplication of elite families via tissue culture appears promising and may be economic, overcoming seasonality problems.
- *Understanding potential for polyploid breeding in* A. mangium *and* A. hybrid *to deliver additional genetic gain.* Field trialling has not progressed sufficiently for any quantitative estimation of gain from deployment of polyploid clones. However during the extension year VAFS obtained a major grant from GoV MARD to continue the program with a focus on triploid clone production and a target of 15ha of pilot plantation trials within 5 years. The project under the leadership of Dr Nghiem Quynh Chi has a budget of $US300,000 and involves a team of 10 staff from VAFS so it represents a major commitment. A peer reviewed paper, by Griffin et al (2015) describes progress to date and lays out a long term polyploid breeding strategy for the future and for consideration by other organisations wishing to explore the potential. We do not know of any other organisations who are yet developing parallel programs but we have had international approaches from organisations interested in trialling triploid clones with proven sterility. These include the US Forest Service in Hawaii. If the current proving trials being conducted by Dr Chi confirm performance and effective sterility of triploid clones, then VAFS is well positioned to licence their germplasm and thus expand the ultimate value created by the Project research.

8.2 Capacity impacts – now and in 5 years

This project takes advantage of the highly developed scientific capability in tree improvement acquired by RCFTI (now IFTIB/VAFS) over the last two decades, and of existing active links with CSIRO and UTas in this field. RCFTI's most experienced scientists will lead research on the Vietnamese side. At the same time at least two younger VAFS scientists will be assigned full-time to the project, developing their research and project management capacity. Two current John Allwright scholars from VAFS will continue their work at UTas on topics closely associated with the project.

IFTIB staff development associated with the project happened as planned. JAF Scholars Mr Tran Duc Vuong (M.Sc) and Dr Nghiem Quyen Chi (Ph.D.) successfully graduated and a third scholar Mr Le Son commenced his Ph.D. program in year 5 with a molecular study of the new population of hybrid clones being screened under Objective 1. Mr Do Huu Son, one of the full time designated project staff from IFTIB is reporting on the project clone trials for his local Ph.D. studies, with advice from Dr Harwood. Mr Vuong is expecting to commence a local Ph.D as part of Dr Chi's new work program. Mr Le Son commenced his Ph.D. program on the development and application of new molecular markers in tropical acacias at UTas late in 2013 and will continue beyond the end of the Project with support of a John Allwright scholarship and additional operating funds provided by ACIAR through the Project. These markers will enhance VAFS ability to genotype selections made in the hybrid breeding breeding program (Objective 1) and further understanding of the complex pedigrees of this material.

The highly successful IUFRO/VAFS Conference "Acacia 2014" (http://iufroacacia2014.com.vn/) was largely an initiative of Project scientists in Vietnam and Australia, with Dr Chi as the local focal point and significant additional support from ACIAR. As far as we are aware such a forestry meeting on this scale had not been run before in Vietnam. The confidence gained, and the international respect earned, should make it easier for VAFS scientists to play a full role in IUFRO and other international science networks in the future.

It should be noted that there are few female scientists in senior positions in VAFS. It seems likely that mentoring by Dr Chi will now have a positive impact in encouraging the development of other young scientists of both genders to undertake training and take their place in the international network of forest science researchers. UTas has recently had an enquiry from a female colleague of Dr Chi's for Ph.D. training financed by a GoV MARD scholarship.

Some training was given in NIR and project scientists in Hobart demonstrated the value of this tool in discriminating between cytotypes (Harbard et al 2014). However VAFS management decided against purchase of the equipment so it was not possible to carry out the planned characterisation of pulp properties of clones in Vietnam.

To progress efficiently with her proposed polyploid breeding program under the MARD grant Dr Chi was in need of a flow cytometer (during the Project this work was done by sending samples to UTas or through expensive and unreliable access to an instrument owned by a 3rd party laboratory in Hanoi). During the extension year ACIAR approved a request to fund purchase of an instrument and for Mrs Price of UTas to visit Hanoi to train two of Dr Chi's staff. A substantial capacity building contribution with immediate benefit.

During the final extension year of the project, the design of the genetic information management system was finalized. The ability to track pedigrees from the initial ATSC seed introductions to current breeding populations and determine relatedness among advanced-generation material, necessary for genetic analysis and planning crossing programs, was required. This was implemented using the database capability of Microsoft Excel. The pedigree databases for the diploid populations of the three commercial acacia species, *A. mangium*, *A. auriculiformis* and *A. crassicarpa* were fully developed and tested and demonstrated to be functional (see Appendix 1). The pedigree databases for the

hybrid clones and for IFTIB's polyploid varieties will be completed and linked to the pure-species databases during 2015-16.

Within Vietnam RCFTI are experienced at managing the transfer of knowledge about use of improved germplasm technology to local nursery and forest extension organizations throughout the country, and through them increasing the value creating ability of tree-growers both large and small. We are confident we can rely on this proven network and experience to disseminate results rather than build new paths to adoption, although a systematic effort will also be made to engage with any other rural development and community forestry projects which have the capability to assist.

Little direct project effort was put into communication with organisations other than IFTIB, as we VAFS has the experience to make sound and appropriate judgements about this. One concrete example was the identification of a commercial nursery partner to test the CFF (Objective 2) methodology for use with *A. crassicarpa* in central Vietnam.

8.3 Community impacts – now and in 5 years

There are some large-scale private-sector forest planting companies, but an estimated 50% of Vietnam's forest planting sector is mainly driven by small-scale (<5 hectares) forest farmers. This means that economic benefits from improved plantation performance are spread more widely throughout rural areas.
The Project will make a positive contribution to the long term viability of the plantation forest industry and hence benefit the communities dependent on this sector. However we cannot claim any direct short term community benefits

8.3.1 Economic impacts

Economic impacts foreshadowed in the Project document related to projected improvements in the growth rates of acacia plantations and in log and wood quality, resulting in higher unit prices to growers and benefits to processing industries. The details of these impact calculations are not repeated here, because circumstances have changed greatly since they were made.

Recent reviews of the prospects for sustainable wood production from acacia and eucalypt plantations in SE Asia (Harwood and Nambiar 2014) and more specifically in Vietnam (Nambiar et al 2015) concluded that three major management inputs: breeding and deployment of improved germplasm; conservative site management that protects and enhances soil and site resources; and protection of the plantation resource against diseases, pests and other threats, must all be brought together in plantation management for enduring success. Thus, the economic benefits of genetic improvement are not guaranteed, but depend on a commitment to sustainable site management and effective risk management (to which genetic improvement contributes).

Over the life of the project, the area of acacia plantations in Vietnam more than doubled, and MARD estimates were that by 2013 the total areas of *A. mangium* and *A. hybrid* plantations had increased to 600,000 and 500,000 ha respectively (Nambiar et al. 2015). This major expansion of acacia forestry and the consequent increase in wood production will increase the benefits obtainable, from any given percentage level of improvement in productivity and log/wood quality that may be obtained from using improved germplasm developed by the project. Recent estimates (N. Byron, pers. comm. 2014) place the current (2013) value of the annual harvest of acacia wood, at the point of entry to processing plants including both local processors and export woodchip mills at over US$700M. An indication of potential project impacts is that a very modest 2% increase in annual acacia log volume production would represent an annual increase in mill-gate log

value of about US$14M. However, realising the economic benefits of improved germplasm from the project will take over a decade, since improved clones or seedlots must be tested by IFTIB, approved by MARD and then deployed widely and grown in operational plantations to harvest age (typically 5-8 years).

It is too early to estimate the economic impacts directly attributable to the Project, but it is already clear that it has positioned VAFS to achieve substantial advances in both deployment of improved germplasm and improved plantation protection. These two outcomes are strongly linked, because deployment of germplasm with increased tolerance of major diseases and pests and climatic stresses will make a crucial contribution to risk reduction. A major expansion of the genetic base of hybrid clones under test, as has been achieved, increases the likelihood of finding such tolerant genotypes.

Assessments of the *A. hybrid* clone trials directly developed by the project under Objective 1 show that at age 2 years there are many clones which display significantly faster growth than the commercial hybrid clones included as controls. IFTIB is now able to test a large number of these fast-growing clones in second-stage clone proving trials that will be established in the major acacia growing regions of Vietnam. This will enable ranking of the new clones for other important traits including stem and branch form, wind and drought tolerance, wood properties, and perhaps most importantly, improved tolerance of diseases such as *Ceratocystis* that are present in Vietnam and have already caused the failure of large areas of acacia plantations in Indonesia and Malaysia (Harwood and Nambiar 2014). Thus, the prospective economic benefits from the project are potentially very large, through increased productivity and log value and also a broader genetic base in which to search for disease and pest resistance.

By 2020 it should be possible to make a realistic assessment of the economic benefits that the first series of “project” clones can bring; by that time productivity of the best clones in large-plot clone proving trials, and their other important traits including disease tolerance will have been determined.

The project has also established effective methods for further large-scale breeding and testing of *A. hybrid* clones, and has developed the resources, notably a new hybridizing seed orchard and the information management system to support this activity.

The prospective economic benefits from mass propagation and deployment of the best families of *A. mangium* and *A. crassicarpa* (Objective 2) are less certain. At this stage, all that can be said is that the project has drawn attention to the desirability of this approach, and has enabled IFTIB to become familiar with a technically feasible method of CFF. It remains to be seen whether the system developed could provide sufficiently high multiplication rates to be economically attractive if applied in production nurseries in southern or central Vietnam.

The polyploid material under development and trial holds the prospect of further qualitative increases in economic benefits, through both trait improvement and the benefits of sterility (lower plantation management costs, and the ability to deploy acacias in invasion-sensitive landscapes, both within and outside Vietnam. Quantitative benefits may also flow if polyploid clones with increased resistance to water stress and/or wind damage result in decreased losses prior to harvest.

8.3.2 Social impacts

The plantation forestry sector is a major employer and is bringing increasing employment and prosperity to many rural areas in Vietnam. Social impacts of the project include:

- *Access to improved germplasm, especially clones, for community growers that want to diversify their forestry interests or are considering acacia-based forestry;*

- *Enhancing the prospect of higher incomes from wood production in the rural sector by contributing to the diversification of production;*
- *Meeting the anticipated strong and increasing demand for wood for sawn timber in Vietnam in the next decade or more;*
- *Reducing reliance on imported roundwood for servicing the requirements (currently 80%) of industries involved in downstream-processing of sawn timber for appearance-grade products;*
- *Stimulating employment and investment into rural areas through growing and utilisation, and service provision, resulting in stimulation of economic activity.*

As for Community benefits (See above) the contribution of the Project is to the long term sustainability, value and profitability of the acacia plantation and processing sector and hence the communities and families dependent upon it. As widely discussed at the Acacia 2014 Conference, acacia plantations are at increasing risk of productivity loss through disease attack and environmental stress. The continued development of genetically diverse improved germplasm that is proven and ready to release to farmers if the current germplasm proves inadequate is a strategically important contribution of the Project.

8.3.3 Environmental impacts

The main perceived environmental problem arising from acacia plantations is the potential for weediness. This is really only an issue for plantations adjacent to uncultivated areas with biodiversity value such as conservation reserves. Acacia plantations make a significant contribution to carbon sequestration at a national level. Increased wood production from acacia plantations in Vietnam, a long-term outcome from this project, should reduce the harvesting pressure on natural forests in Vietnam and other countries. Currently Vietnam imports about 80% of the wood used by its solid-wood processing industries, and a substantial proportion of this imported wood is not sustainably harvested.

Development of triploid production clones as a direct result of this Project, either infertile or with greatly reduced fertility, would bring significant benefit for planting areas where weediness is a potential problem (e.g. adjacent to national parks or other native vegetation susceptible to invasion). Use of infertile clones, would also reduce the germination of wildlings during replanting and stand establishment, simplifying management of successive acacia plantations or conversion of plantations to other land uses.

8.4 Communication and dissemination activities

Data from the project website Breeding Tropical Acacias http://www.breedingtropicalacacias.com/page1.php for 2014 show 19,134 unique visitors averaging 4 visits each. There was a significant increase in visitor numbers to 3003 in May 2014 from a previous average of 295 monthly visitors. This level of interest can be attributed to the “Acacia 2014” Hue, Vietnam conference and the interest was maintained to the end of 2014 with monthly visitor numbers averaging 2129.

An additional review publication which is widely cited was made possible through time devoted to the Project. Griffin et al (2011) Global uses of Australian acacias—recent trends and future prospects. Diversity and Distributions, 17, 837-847 has been cited 29 times

In summary, a total of 16 peer reviewed papers have been published by project scientists (see section 9.3) with 6 more papers in preparation or planned for production. A VAFS scientist was senior author on 6 of these papers. Project work was also extensively

reported to international audiences of over 150 people in (i) 3 talks at the IUFRO Forest Biotechnology conference in Kuala Lumpur, in March 2010 (ii) a talk at the Australasian flow Cytometry conference, Hobart 2011 and (iii) a series of 7 posters and 3 invited talks at the IUFRO/VAFS Conference “Acacia 2014” at Hue in March 2014.

9 Conclusions and recommendations

The overarching aim of the Project was to assist VAFS strengthen their acacia tree improvement programs, especially in relation to *A. hybrid*, by bringing a new level of sophistication to both approach and technologies in each of the 3 major research areas. These work programs were executed substantially according to plan, in some cases with additional value added through activities which were not originally scheduled. All project scientists from both Vietnam and Australia showed an exceptional level of dedication and professionalism in addressing the difficult task of collaborating on a sustained basis “at a distance”. Through conservative management of resources we were able to fund a no-cost extension which allowed completion of a number of important lines of investigation and assisted VAFS staff to successfully gain GoV support to build on the foundation laid by the Project. Key achievements were:

Objective 1. Hybrid clonal program.

The project has achieved its aim of positioned VAFS to deliver large economic benefits to Vietnamese growers through the deployment of a new generation of fully tested hybrid clones. The staged evaluation of over 5000 hybrid seedlings with clonal testing of the best 550 permits VAFS to advance to wider field testing of the very best clones in 2016. Superior, well-tested new clones derived from a broad genetic base could, in principle, be tested for MARD approval and subsequent commercial deployment by 2020 or soon thereafter. Many of the new clones equal or exceed the early growth of the current commercial hybrids so we are confident that benefits will be realised

The Project has also contributed to the next generation of hybrid breeding through installation of a new seed orchard containing the best 10 clones of *A.mangium* and of *A.auriculiformis* in the pure species breeding programs and has secured ability to track pedigrees in these increasingly complex breeding programs through development of an new genetic information system.

Objective 2 – CFF propagation to expand utilisation of tested seed orchard seed of *A. mangium*

The development of methodology based on experience in Indonesia was successfully accomplished. However at the location chosen in northern Vietnam the growing season was not long enough to deliver economically viable multiplication rates. Early results from a field trial indicate that growth and survival of CFF cuttings is indistinguishable from that of seedlings of the same *A. mangium* families so that this is no impediment to uptake of the technology by VAFS if they can identify a situation in more southerly areas where the growing season is longer**.**

Objective 3 –Continued development of new polyploid varieties

The Project work program demonstrated many differences between polyploid and diploid cytotypes, thus achieving one important aim of expanding the effective genetic diversity available to breeders of the core species *A.mangium.* It is clear that 4x trees have different wood properties that are potentially of commercial value and that 3x trees show much reduced fertility and thus reduced potential for weediness. However the big unknown is volume production. All the neo-tetraploids we have produced grow more slowly than diploids at least in the early years. We do not yet have trial data about growth rate or other production attributes of triploids although the superior growth of the one spontaneous triploid found in progeny of a 4x mother is encouraging and early observations of recent trials by VAFS have identified two 3x clones which are growing at least as fast as commercial hybrid controls. For planting in ecologically sensitive areas it will only be necessary for a triploid clone to be comparable in production to a diploid alternative to make an operational option. Elsewhere growers will need to be convinced that the ultimate value of their crop will be higher if they plant polyploid clones. If they

need to be grown on a longer rotation to achieve comparable volume then they will only be planted if there is a direct price premium for superior wood properties, or they have a substantially higher probability of producing a crop through superior stress adaptation, or resistance to wind damage pest and diseases. The case can only be made on the basis of field trial results and effective demonstration plantings to convince farmers. The recent MARD grant to Dr Chi of VAFS will permit great progress with this testing during the next 5 years. That time will also be sufficient for indicative results regarding the postulated growth advantage of new highly heterozygous polyploid clones.

Capacity Building: In addition to the scientific achievements, Project managers are proud of the capacity building outcomes which will have lasting benefit for VAFS. Scientists trained under the John Allwright Fellowship are functioning as increasingly effective researchers within IFTIB. New instrumentation (CyFlow Ploidy Analyser) and software (the genetic information management system) developed through the Project are now in use. Most importantly, through engagement in running the highly successful international Conference “Acacia 2014”, VAFS staff have established a confident position in the regional network of forest scientists. Hopefully this will contribute to a more collaborative approach to research on the many issues of common interest beyond the national boundaries of Vietnam.

9.1 Recommendations

There will be an ongoing demand for investment in genetic improvement of acacia in Vietnam. This is recognised by GoV and VAFS staff are now well equipped to implement sound strategic plans. However new challenges face the breeders, in particular determining response to the increased incidence of diseases identified in the “Acacia 2014” Conference. A strong recommendation from the Conference was that a cross disciplinary approach was required for future R&D. Geneticists, pathologists and silviculturists need to work together to develop an integrated package of management prescriptions which will ensure a sustainable supply of high quality acacia wood and return an adequate profit to the farmer producers. ACIAR is well placed to encourage and support projects which are framed with the principle in mind.

The efficient commercial exploitation new polyploid varieties present as yet unknown opportunities / challenges. Planning to address such issues would undoubtedly benefit from continued ability of Vietnamese and Australian scientists to interact. If ACIAR can find the means to facilitate this active networking benefits would flow not only to Vietnam but also to other countries in the region with significant investment in acacia plantation forestry.

9.2 References cited in report

Technical Reports and conference poster presentations are available on the Project website http://www.breedingtropicalacacias.com/. These are available for download from the Members area / Documents Library. For access click on the link provided and select ‘password request’.

Blakesley D, Allen A, Pellny TK, Roberts AV. 2002. Natural and induced polyploidy in *Acacia dealbata* Link. and *Acacia mangium* Willd. Ann Bot (Lond) 90: 391-98

Brawner J, Japarudin Y, Lapammu M, Rauf R, Boden D, Wingfield MJ. 2015. Evaluating the inheritance of *Ceratocystis acaciivora* symptom expression in a diverse *Acacia mangium* breeding population. Southern Forests: a Journal of Forest Science 77: 83-90

Bretagnolle F, JD T. 1995. Gametes with the somatic chromosome number: mechanisms of their formation and the role in the evolution of autopolyploid plants. New Phytologist 129: 1-22

Do Huu Son, Le Son, Chris Harwood, Thinh HH. 2014. Development and testing of Acacia hybrid clones in Vietnam. In "Acacia 2014": http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Do_Huu_Son_et_al_Presentation_to_Acacia2014_Development_and_testing_of_Acacia_hybrid_clones_in_Vietnam.pdf

Drs R Griffin, C Harwood, Clarke C. 2013. “Towards commercial deployment of polyploid Acacia in Vietnam” Working Paper prepared for VAFS and ACIAR with inputs from colleagues in VAFS, IFTIB. with an annotation by RG in April 2014, http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Griffin_et_al_Commercialisation_Study_Report_August_2013.pdf

Griffin AR, Nambiar EKS, Harwood CE, See LS. 2015. Sustaining the future of Acacia plantation forestry – a synopsis. Southern Forests: a Journal of Forest Science 77: v-viii

Griffin AR, S.J. Midgley, D. Bush, P.J. Cunningham, A.T. Rinaudo. 2011. Global uses of Australian acacias—recent trends and future prospects. Diversity Distrib 17: 837–47

Griffin AR, Vuong TD, Vaillancourt RE, Harbard JL, Harwood CE, et al. 2012. The breeding systems of diploid and neoautotetraploid clones of *Acacia mangium* Willd. in a synthetic sympatric population in Vietnam. Sexual Plant Reproduction 25: 257-65

Griffin AR, Twayi H, Braunstein R, Downes GM, Son DH, Harwood CE. 2014. A comparison of fibre and pulp properties of diploid and tetraploid *Acacia mangium* grown in Vietnam. Appita 67: 43-49

Ha Huy Thinh, Le Dinh Kha, Phi Hong Hai, Do Huu Son, Nghiem Quynh Chi, et al. 2014. Vietnam’s acacia breeding strategy. In "Acacia 2014". http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Ha_Huy_Thinh_et_al_Presentation_to_Acacia2014_Vietnam___s_acacia_breeding_strategy.pdf

Harbard JL, Lee Chang KJ, Son Du Huu, AR G. 2014. Total phenol concentrations of bark extract from a diploid and autotetraploid *A. mangium* population in Vietnam. Technical Report http://www.breedingtropicalacacias.com/page20.php

Harbard J, Nghiem Q. 2014. Production of open pollinated triploid Acacia in a polyploid hybridising orchard in Vietnam and early data on triploid sterility. In Acacia 2014 "Sustaining the Future of Acacia Plantation Forestry". Hue, Vietnam http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Harbard_and_Chi_Presentation_to_Acacia2014_Production_of_3x_Acacia_in_a_polyploid_hybridising_orchard_in_Vietnam_and_early_observations_of_sterility.pdf

Harbard JL, Griffin AR, Foster S, Brooker C, Kha LD, Koutoulis A. 2012. Production of colchicine-induced autotetraploids as a basis for sterility breeding in *Acacia mangium* Willd. Forestry 85: 427-36

Harwood CE, Hardiyanto EB, Yong WC. 2015. Genetic improvement of tropical acacias: achievements and challenges. Southern Forests: a Journal of Forest Science 77: 1-8

Jane L Harbard, Aina Price, Tran Huu Bien, Rodemann T. 2014. Discrimination between autopolyploids and diploid Acacia using near-infrared reflectance spectroscopy. Presented at "Acacia 2014", Hue, Vietnam http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Har

bard_et_al_Presentation_to_Acacia2014_Discrimination_between_autopolyploids_and_diploid_Acacia_using_NIR_reflectance_spectroscopy.pdf

Kha LD. 2001. Studies on the use of natural hybrids between *A. mangium* and *A. auriculiformis* in Vietnam. Hanoi: Agriculture Publishing House.

Kha LD, Harwood CE, Kien ND, Baltunis BS, Hai ND, Thinh HH. 2012. Growth and wood basic density of acacia hybrid clones at three locations in Vietnam. New Forests 43: 13-29

Lam HK, Harbard JL, Koutoulis A (2014) Tetraploid induction of *Acacia crassicarpa* A.Cunn. Ex Benth. using colchicine and oryzalin. Journal Tropical Forest Science 26:347-354.

Nambiar EKS, Harwood CE. 2014. Productivity of Acacia and Eucalypt Plantations in Southeast Asia. 1. Bio-Physical Determinants of Production: Opportunities and Challenges. Int Forest Rev 16: 225-48

Nambiar EKS, Harwood CE, Kien ND. 2014. Acacia plantations in Vietnam: research and knowledge application to secure a sustainable future. Southern Forests: a Journal of Forest Science: 1-10

Nghiem CQ, Harwood CE, Harbard JL, Griffin AR, Ha TH, Koutoulis A. 2011. Floral phenology and morphology of colchicine-induced tetraploid *Acacia mangium* compared with diploid A*. mangium* and *A. auriculiformis*: implications for interploidy pollination. Australian Journal of Botany 59: 582-92

Nghiem Q, Harbard JL, Harwood CE, Griffin AR, Ha TH, Koutoulis A. 2013. Pollen–pistil interactions between autotetraploid and diploid *Acacia mangium* and diploid A. *auriculiformis*. Journal of Tropical Forest Science 25: 96-110

Nghiem QC, A.R. Griffin, C.E. Harwood, J.L. Harbard, Ha H.T., Koutoulis A. 2015 (submitted). Seed development following reciprocal crossing among autotetraploid and diploid *Acacia mangium* Willd. and diploid *A. auriculiformis* A. Cunn. ex Benth.

O'Grady AP, Pinkard EA, Mitchell PJ, Harbard JL, Price, AJ, Griffin AR Comparative ecophysiology of Acacia hybrid clones, does polyploidy infer increased drought tolerance? (in prep)

Paul Warburton, Mai Trung Kien, Bui Tien Hung, Do Huu Son, Duong Thanh Hoa, Harwood C. 2014. Evaluating Clonal Family Forestry as a deployment strategy *for Acacia mangium* in Vietnam POSTER. Presented at "Acacia 2014", Hue, Vietnam http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Warburton_etal_Presentation_to_Acacia2014_Evaluating_Clonal_Family_Forestry_as_a_deployment_strategy_for_A_mangium_in_Vietnam.pdf

Price A. 2011. Micropropagation and Tetraploid Induction Report: *Acacia mangium/Acacia auriculiformis* hybrid clones Technical Report, http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/Objective_3/Price__Acacia_hybrid__micropropagation___tetraploid_induction_May_2011.pdf

A.J. Price, L. Son, J.L. Harbard, A.R. Griffin and A. Koutoulis 2014 Ploidy stability in hedge plants of neo-tetraploid Acacia hybrid clones POSTER Presented at "Acacia 2014", Hue, Vietnam http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/GenDocs/Price_et_al_Presentation_to_Acacia2014_Ploidy_stability_in_hedge_plants_of_neo-tetraploid_Acacia_hybrid_clones.pdf

Richardson DM, Le Roux JJ, Wilson JRU. 2015. Australian acacias as invasive species: lessons to be learnt from regions with long planting histories. Southern Forests: a Journal of Forest Science: 1-9

Vuong Tran Duc (2010) Microsatellite analysis of seed production systems in Acacia mangium. Research Master thesis, University of Tasmania

Warburton P. 2014. Evaluating Clonal Family Forestry as a deployment strategy for *Acacia mangium* in Vietnam FINAL REPORT, http://www.breedingtropicalacacias.com/attachments/File/Links/Documents/Objective_2/Warburton_Evaluating_Clonal_Family_Forestry_as_a_deployment_strategy_for_Acacia_mangium_in_Vietnam__FINAL_2014.pdf

White TL, W. T. Adams, Neale DB. 2007. Forest genetics. Wallingford, United Kingdom: CAB International.

Wong CY. 2010. Capturing fiber gain of *A. mangium* and *A. crassicarpa* through clonal family forestry. Presentation at IUFRO Conference in Forest Biotechnology, Kuala Lumpur, March 2010.

Wong CY, Haines RJ. 1992. Multiplication of Families of *Acacia mangium* and *A auriculiformis* by Cuttings from Young Seedlings. Breeding Technologies for Tropical Acacias 37: 112-14

9.3 List of peer reviewed publications produced by project

1. Le Dinh Kha, R. Griffin, Ha Huy Thinh, J. Harbard, Le Son, Duong Thanh Hoa, Nghiem Quynh Chi (2009) Research on ploidy identification and characterisation of *A.mangium* in comparison with diploid clones in Vietnam. Science and Technology Journal of Agriculture & Rural Development. No 9. pp. 114-120. (Vietnamese with English Summary)

2. AR. Griffin, Tran Duc Vuong, J. L. Harbard, C. Y. Wong,C., Brooker, R. E. Vaillancourt and L.D.Kha (2010) Improving controlled pollination methodology for breeding *Acacia mangium* Willd. New Forests 40: 131-42

3. Tran, Duc Vuong (2010) Microsatellite analysis of seed production systems in Acacia mangium.Research Master thesis, University of Tasmania

4. Le Dinh Kha, C.E. Harwood. Nguyen Duc Kien; B.S. Baltunis, Nguyen Dinh Hai and Ha Huy Thinh (2012) Growth and wood basic density of acacia hybrid clones at three locations in Vietnam. New Forests 43:13–29

5. C.Q. Nghiem, C.E. Harwood, A.R. Griffin, J.L. Harbard, A. Koutoulis and T.H. Ha, (2011). Floral phenology and morphology of colchicine-induced tetraploid *Acacia mangium* compared with diploid *A. mangium* and *A. auriculiformis* in relation to inter-ploidy pollination. Australian Journal of Botany 59(6): 582-592

6. A.R. Griffin; S.J. Midgley; D. Bush; P.J. Cunningham and A.T. Rinaudo (2011) Global uses of Australian acacias - recent trends and future prospects. Diversity and Distributions, 17, 837-847

7. Harbard J. L., Griffin A.R., Foster S., Brooker C., Koutoulis A., and, L. D. Kha. (2012) Colchicine-induced chromosome doubling as a basis for polyploid breeding in *Acacia mangium* Willd. Forestry 85 (3): 427-436

8. Griffin AR, Vuong TD, Vaillancourt RE, Harbard JL, Harwood CE, Nghiem CQ, Thinh HH (2012) The breeding systems of diploid and neoautotetraploid clones of *Acacia mangium* Willd. in a synthetic sympatric population in Vietnam. Sexual Plant Reproduction 25, 257-265.

9. Nghiem Q, Harbard JL, Harwood CE, Griffin AR, Ha TH, Koutoulis A (2013) Pollen–pistil interactions between autotetraploid and diploid *Acacia mangium*

and diploid *A. auriculiformis*. Journal of Tropical Forest Science 25, 96-110.

10. Nghiem Quynh Chi (2013) Effects of ploidy level on the reproductive biology of tropical *Acacia* species PhD thesis, University of Tasmania

11. A.R. Griffin, H. Twayi, R. Braunstien, G.M. Downes, Do Huu Son and C.E. Harwood (2014) A comparison of fibre and pulp properties of diploid and tetraploid *Acacia mangium* grown in Vietnam. Appita 67 (1): 43-49

12. Lam HK, Harbard JL, Koutoulis A (2014) Tetraploid induction of *Acacia crassicarpa* A.Cunn. Ex Benth. using colchicine and oryzalin. Journal Tropical Forest Science 26:347-354.

13. Harwood CE, Hardiyanto EB, Yong WC. 2015. Genetic improvement of tropical acacias: achievements and challenges. Southern Forests: a Journal of Forest Science: 1-8

14. Griffin AR, Chi NQ, Harbard JL, Son DH, Harwood CE, et al. 2015. Breeding polyploid varieties of tropical acacias: progress and prospects. Southern Forests: a Journal of Forest Science: 1-10

15. Griffin AR, Nambiar EKS, Harwood CE, See LS. 2015. Sustaining the future of Acacia plantation forestry – a synopsis. Southern Forests: a Journal of Forest Science 77: v-viii

16. Nambiar EKS, Harwood CE, Kien ND. 2014. Acacia plantations in Vietnam: research and knowledge application to secure a sustainable future. Southern Forests: a Journal of Forest Science 77: 1-10

In preparation.

Price A.J., Koutoulis A. Invitro production and identification of colchicine-derived tetraploid Acacia hybrid clones. (in prep)

O'Grady AP, Pinkard EA, Mitchell PJ, Harbard JL, Price, AJ, Griffin AR Comparative ecophysiology of Acacia hybrid clones, does polyploidy infer increased drought tolerance? (in prep)

Do Son and Harwood CE Selecting acacia hybrid clones in Vietnam: stability of performance across contrasting environments (in prep)

Nghiem CQ and Harbard.JL Detection and early observations of sterility of triploid tropical Acacias. (in prep)

Harbard JL and Griffin AR Fibre properties of diploid and autotetraploid tropical Acacia Hybrid (*A mangium* x *A auriculiformis*) (in prep)

Son Le, Wickneswari Ratnam, Christopher Harwood, Rod Griffin, Anthony Koutoulis, Jane Harbard, Matthew Larcombe, Maid Mandy, Koh Sin Cyer, Liew Wai Yee, Thinh Huy Ha and René E. Vaillancourt Molecular markers for population genetic studies, discrimination of pure species and hybrids between *Acacia mangium and A. auriculiformis (in prep)*

10 Appendixes

10.1 Appendix 1: Pedigree management for acacia species in IFTIB tree breeding programs

Pedigree management for acacia species in IFTIB tree breeding programs

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Background: IFTIB's acacia breeding programs

IFTIB's acacia breeding programs commenced from introductions of provenance bulk and individual-family seed collections of *A. auriculiformis*, *A. crassicarpa* and *A. mangium* (AA, AC and AM) most of these originating from CSIRO's Australian Tree Seed Centre (ATSC). Introductions commenced in the late 1980s and continue to this day. The main base populations of individual open-pollinated (OP) families were introduced in the mid-1990s (AA and AM) and in the early 2000s (AC). Many of the families introduced to Vietnam originated from the wild provenances, but a considerable number were families collected from superior parent trees in seed orchards established outside the species' natural ranges. Families from seed orchards of AA in Thailand and the Northern Territory, Australia, and seed orchards of AM in northern Queensland, Australia contributed substantial proportions of the genetic base of these species. The genetic composition of these out-of-country seed orchards is known.

IFTIB has conducted provenance trials and first-generation provenance/progeny trials of each of the three species. Some trials have been converted into seed production areas and seed orchards. Superior trees have been identified and seed collected from these for establishment of second generation progeny trials. Many of the second-generation progeny trials in fact test both these local selected families and additional first-generation families or provenances. For AA, there has also been substantial testing of selected clones.

IFTIB also has a breeding program for the acacia hybrid between *A. mangium* and *A. auriculiformis* to deliver superior, tested individual hybrid clones. The original acacia hybrid clones planted commercially in Vietnam since the late 1990s were spontaneous hybrids identified in *A. mangium* plantations of known provenance origin (Kha et al. 2012) but new hybrid candidates are now being identified from more structured populations such as "hybridizing orchards" where maternal parent identities, at least, are known. Some new hybrid candidates are being derived through controlled pollination.

In 2003 IFTIB also commenced a polyploid breeding program, summarized by (Griffin et al. 2015). Polyploids of all three species and the interspecific hybrid have been developed, and crossing between them has commenced.

Need for managing pedigree information

One of the sub-objectives in ACIAR Project FST/2008/07 was to develop an information management system to support acacia hybrid breeding. It is important to maintain pedigree information over successive generations of acacia breeding as far as possible, for several reasons:

1) It is known that inbreeding depresses vigour of the resulting progeny in these acacia species. (Harwood et al. 2004) showed that for AM, progeny arising from self-fertilization grew much more slowly than outcrossed progeny. Selfing is the most extreme form of inbreeding. Mating between close relatives is a less severe form of inbreeding, but is expected to result in some decline in vigour, compared with mating between unrelated individuals. The extent of relatedness should be considered when planning crossing programs or seed orchard composition. For example, in a controlled crossing program, we might wish to avoid breeding between two closely related individuals, both descended from the same grandmother tree.
2) Alternatively, we might find that a particular genotype performs well as a mother or a father in a controlled crossing program, and might wish to identify and select other, related, individuals which might share this good performance, for use in future crossing.
3) When selecting the next generation of a breeding population, or the composition of a seed orchard, we want to know how much genetic diversity we are carrying forward, and how much we are losing. We might be concerned that the genetic base in advanced generations of the breeding population is becoming too narrow, leading to reduced genetic variation in traits such as wind or disease resistance and therefore an inability to select and breed under changing circumstances. When adding genotypes to advanced-generation breeding populations (infusion populations), we need to know the relationships between the infusion population and the existing population. To do this we need to examine their pedigrees, back to the original wild provenance and mother trees. Infusion populations may be from breeding programs in other countries, which started with a base of some or all of the same wild populations as did IFTIB.
4) To correctly analyse advanced-generation genetic trials, it is necessary to know the genetic relationships among treatments (for example among OP families). We might test 100 open-pollinated families in a series of progeny trials, but if all these families are descended from only 50 maternal grandparents, they are more closely related than we would otherwise assume. If we know the pedigrees of the 100 families we can use a pedigree file when carrying out a genetic analysis, which will enable us to estimate the correct values of genetic parameters such as heritabilities and genetic correlations that are used to predict genetic gain, and correctly calculate breeding values that are used to guide selection of individuals for subsequent breeding.

Systems for genetic information management

Comprehensive systems for genetic information management have been developed. For example, the Southern Tree Breeding Association, a breeding cooperative in Australia, has developed TreePlan software for genetic analysis, which depends on DataPlan, a purpose-designed relational database for genetic information management that maintains the data and genetic information for hundreds of thousands of trees of two species (*Eucalyptus globulus* and *Pinus radiata*) in hundreds of field trials belonging to STBA members. However, it would be very expensive for IFTIB to purchase access to TreePlan and DataPlan, and their use would require expensive ongoing consultant services from STBA experts and training of IFTIB staff in its use. Dr Stephen Verryn of Creation Breeding in South Africa is currently developing a similar system named TreePlasm, but similar considerations of cost and reliance on consultants would apply. These systems are based on relational databases which assign a stem identity and genetic identity to every tree in every genetic trial of the breeding program – for example if there were 100 trials with an average of 1000 stems in each trial, there would be over 100,000 entries in the database.

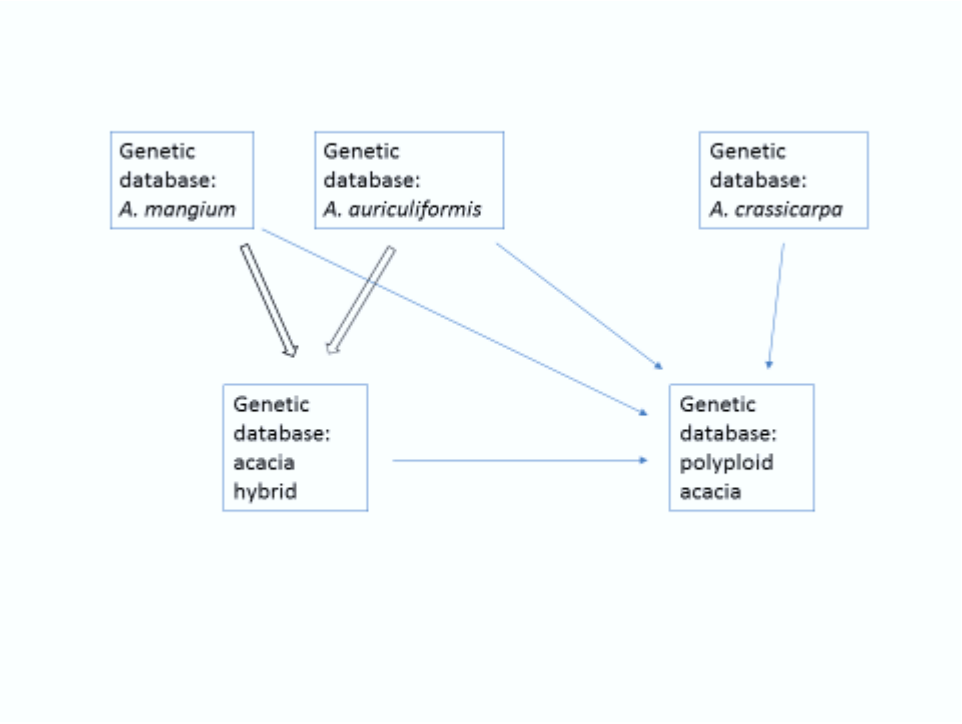
The alternative is to develop a simple, in-house database for basic pedigree management, using Microsoft Excel. This database functions at the family level and does not maintain

records for each individual tree, although it does record where each seed family originates and is tested. This will provide simplicity and ease of use, consistent with IFTIB's pedigree management requirements, and should be usable by IFTIB staff without reliance on external consultants. This is the option that has been developed. Acacia breeding in Vietnam is mostly by open-pollination, so in most cases pedigree information on the male side is not available. In each generation, all that is known on the male side is the pollen pool (for initial introductions, this is the wild provenance or ex-situ seed orchard, and in subsequent generations in Vietnam it is the trial or seed orchard within which a selected seed tree is located). There is no need to identifying pollen collections as separate genetic identities, because pollen collections for controlled pollination are not stored long-term.

For each of the three acacia species, to date only a few hundred open pollinated families are under test by IFTIB. For AA and acacia hybrid, there are also a few hundred clones under trial. There are currently about 15-30 field trials per species (about 30 for each of AM and AA, 13 for AC). This enables all the genetic information for a species, at the family level, to be managed in an excel spreadsheet sized at a few hundred rows x 30 columns. Once set up in the spreadsheet, queries concerning relatedness among selections can be made using Excel's Pivot table, Sort and V-lookup functions, as shown below. Pedigree files for genetic analysis of individual field trials or trial series can be readily constructed from the Excel pedigree database, as required for particular genetic analyses.

Recommended system

The recommended system is comprised of 5 separate Excel pedigree databases, as shown below



There will be five separate pedigree databases, represented on five separate Excel spreadsheets, as shown in Figure 1. The AA and AM databases are the foundations for the acacia hybrid database. All hybrid individuals have a pedigree involving provenances, families or trees that will be represented in one or both of the AM or AA databases. The polyploid acacia database is built up primarily from individuals with pedigree information coming from the other four databases, as shown by the arrows, although increasingly there will be open pollination or crossing within the polyploid populations.

Later, it may be possible to combine all 5 databases into one larger database, but the first step has been to get them functioning separately.

Setting up the pedigree databases: example of *A. crassicarpa* (file crassicarpa pedigree database Dec 24 2014.xlsx)

Each row in the pedigree database is a unique genetic identity (GeneID).

Genetic identities are each given a unique alphanumeric code, and are identified as a type, which in most cases is either a provenance, a seed orchard overseas, stand in Vietnam (local pollen pool), a seed family, a tree, or a clone.

The steps are as follows:

1. List all imported provenances of *A. crassicarpa* and give them a supplier GeneID. In most cases this will be the ATSC provenance collection number

   Example: ATSC16597 is *A. crassicarpa* provenance collection from Gubam Village, PNG

2. Then list all individual mother trees which yielded open-pollinated family seedlots in the imported family collections, using the collector tree ID (for ATSC family seedlots, this is a unique seed collector code which identifies each collected seed tree).

   Example: BVG1108, which is a seed tree in Gubam Village, PNG provenance. This tree was collected, giving an individual seed family, as part of the ATSC provenance collection ATSC 16597 at Gubam Village, PNG provenance.

   Note: the worksheet "unique GeneIDs 1st gen mums" shows the provenance names associated with the wild mother tree GeneIDs

3. Then create open-pollinated family seedlot GeneIDs corresponding to each of the imported family seedlots from mother trees in step 2. Give them each unique GeneIDs in the format AC0001, AC0002, AC0003, etc.
   Example: Open-pollinated family seedlot AC0012 is the family seedlot collected from tree BVG1108, which is a mother tree in Gubam Village, PNG provenance ATSC 16597 and was collected by Brian Gunn.

   Note: It may seem unnecessary extra work to distinguish between the mother tree BVG1108, located in Gubam provenance, PNG, and the seedlot from it, tested in Vietnam, AC0012. But this is an important distinction (they are two separate generations!) and it is a necessary distinction for pedigree-based genetic analysis. You will see that seedlot AC0012 has as its mother, tree BVG1108, and the "father" side is listed as ATSC16597, the Gubam PNG provenance. This provenance provided the pollen pool for the OP family seedlot that was collected from tree BVG1108.

4. The first 5 columns in the pedigree database are the GeneID (coloured red), the mother ID, the father ID, the type (tree, OP family, provenance, Vietnam stand, clone) and the location (wild, plantation, trial, etc.) Location is important for helping us to keep track of where individual GeneIDs are located. In the base population (founder generation), most father IDs are natural provenance pollen pools

5. The next 13 columns are for the IFTIB field trials where GeneIDs are tested. The field codes used are shown in these columns, for each GeneID that has been tested in field trials in Vietnam. So you can look down each column and easily see all the GeneIDs tested

in each trial. Use Data/Filter to get a consolidated list for each trial. Field trials can (and in some cases do) test provenances, OP families and clones together in the one trial. Ideally, for a single GeneID we should use the same field code in all field trials. For *A. crassicarpa*, some but not all GeneIDs use a single field code. By looking across the rows you can see all the trial locations where each GeneID has been tested

Example: OP family AC0112 (collected from seed tree BVG2792 in ATSC provenance ATSC18937) has been tested in 7 IFTIB field trials (trials 1, 2, 3, 4, 8, 9 and 10). It was given the field treatment code 32 in trial 1, the code 64 in trials 2 and 3, and the code 4 in the other four trials. When doing across-site analysis of these trials it would be necessary to re-assign field codes for AC0112 to make them the same across all seven trials.

6. Once all "wild" original OP families tested by IFTIB have been assigned GeneIDs, we allocate unique GeneIDs to those trees in the first-generation field trials that have been selected for seed collection or cloning. If a selected tree is from a known OP family, the maternal parent will be shown as the mother ID for that selection, while the father ID will be the pollen pool around that mother tree. Example: GeneID AC0408 is a tree selected from the first-generation progeny trial at Ham Thuan Nam. In that trial it had field code 64. The selected tree was the first tree in replicate number 2 in the trial. This location is shown in columns T to X, on the right of the spreadsheet. Note that it has exactly the same mother ID (BVG2792) and father ID (ATSC18937) as does OP family seedlot AC0112, of which it is a member. But it is a selected tree, not an OP family seedlot, so it is given its own unique GeneID.

7. Then we allocate unique GeneIDs to the seed families (almost always OP) collected from the selected trees in step 6. Again, this may look like unnecessary work but is a crucial step because the seed family is the next generation on from the selected tree. The mother ID for each of these selected families is the GeneID of the selected seed tree, while the father ID is the pollen pool for the field trial where the mother occurs.

   Example: GeneID AC0588 is the OP family collected from the selected tree with GeneID AC0408 (see step 6 above). So its mother ID is AC0408 and its father ID is the Ham Thuan Nam seed orchard pollen pool. This OP family is tested as experimental treatment 34 in progeny trial 7 planted at Ham Thuan Nam in 2011 (see column L)

8. To avoid confusion, the 13 *A. crassicarpa* clones planted in the clone bank and Bau Bang were allocated unique GeneIDs AC1 to AC13, which were the codes used in the field planting. Note: AC1 is a different unique GeneID to AC0001

Steps 6 and 7 can be repeated for each new "round" of tree selection and seed collection from the selected trees, carried out by IFTIB. I have called these "rounds" rather than generations because generations soon get blurred and we have instead a multi-generation "rolling front" of selection and seed collection followed by progeny trial establishment.

General note on numbering GeneIDs.

It appears best to set up the pedigree database for each pure species in the sequence outlined above. When allocating GeneIDs AC0001, AC0002, etc, it is best at the end of each step to leave some (about 50) unallocated GeneID numbers before choosing the first

GeneID number for the next step. Then additional numbers in sequence can be allocated when additional families or selections are made.

Example: tree AC0431 is the last tree, so far, selected in first-generation trials. There is a gap to the next GeneID AC0450 which is the first OP family from a selected tree. A bigger gap could be left.

Some of the *A. crassicarpa* GeneIDs are non-sequential, for example the wild provenances (ATSC16593, etc.) wild trees (BVG1105, etc.) and the 13 clones (AC1 to AC13). This is to make use of well-used unique numbers that can function as GeneIDs. However, it is necessary to check that there is no overlap so that no two entities (rows in the database) have the same GeneID. This can be checked using the DataTable function in Excel.

Security

Once we have set up the databases it is essential that a stable version be maintained. The database should be password-protected with only Ms Hoa able to modify it (other people can work with copies, for their own purposes such as genetic analysis). Hoa would make written notes every time she makes a change or adds more GeneIDs. The notes can be kept on a separate worksheet of the Excel pedigree database file labelled "*notes*"

Using the pedigree database: examples

Example 1. Checking relatedness among IFTIB's selected trees.

See worksheet "second gen relatedness" on the pedigree database

Copy the GeneIDs and motherID columns for all trees selected by IFTIB to a separate worksheet. Set up a data table query on this block of two columns and 188 rows (selected trees), as shown. You can see that the 188 selections have come from only 88 original maternal GeneIDs (wild provenance or OP family or local SPA). Some of the wild OP families have contributed more than three selected trees. For example OP family AC0013 from collected tree BVG1109 (ATSC 16597, Gubam Village PNG provenance) has contributed **six** selected trees identified in first-generation progeny trials, from which OP family seedlots have been collected. Most breeding strategies do not recommend selecting and testing any more than **three** OP seed families, at maximum, descended from one original OP family. The reason for this is that the top three trees from a good OP family are likely to have very similar genes and yield OP families with similar performance. Making a further three selections from the same OP family is unlikely to deliver additional genetic diversity or genetic gain. Note that if the different trees come from different trials with different pollen pols, then it is more likely that their OP progeny will contain additional genetic diversity. So if selecting two or more trees from an OP family, try to select from two or more different trials where the family is tested.

You can see the narrowing of the genetic base as we move from the initial provenances and families to the first-generation selections. There were 26 provenances and 207 wild families, and selections from the Ba Vi SPA represented in first-generation IFTIB trials, and only 88 of these (8 provenance bulk seedlots and 79 OP families, plus the Ba Vi SPA) are represented among the 188 selections from first generation trials. This is a reasonable degree of narrowing – we need to select among provenances and families to make genetic gain – but narrowing of the genetic base needs to be kept under review as we proceed to advanced generation selections.

Example 2. Planning crossing programs – taking into account relatedness

If we were planning a CP crossing program among the 13 *A. crassicarpa* clones in the clone bank at Bau Bang, we could look at their pedigrees.

GeneID	Mother ID	Father ID
AC1	ATSC20141	ATSC20141
AC2	BVG2758	ATSC18940
AC3	BVG2758	ATSC18940
AC4	ATSC19731	ATSC19731
AC5	BVG01125	ATSC16597
AC6	MM1289	ATSC17849
AC7	BVG01127	ATSC16597
AC8	ATSC19739	ATSC19739
AC9	BVG01125	ATSC16597
AC10	MM1289	ATSC17849
AC11	ATSC19685	ATSC19685
AC12	BVG2732	ATSC18940
AC13	BVG01108	ATSC16597

Clones AC2 and AC3 both come from the same original mother tree, BVG2758 in provenance ATSC18940, Bimadebun Provenance, PNG). If we choose to cross these two we would be crossing closely related trees than have the same mother (tree BVG2758) and possibly the same father. Whereas a cross between AC1 and AC2 is a cross between two completely unrelated trees from two different provenances, even though we don't know the actual mother tree for AC1 since it comes from a bulk seedlot (ATSC20141, Trans Fly PNG).

Example 3. Across-site genetic analysis using pedigree files

This will be completed later.

References

Griffin, A. R., N. Q. Chi, J. L. Harbard, D. H. Son, C. E. Harwood, A. Price, T. D. Vuong, A. Koutoulis, and H. H. Thinh. 2015. Breeding polyploid varieties of tropical acacias: progress and prospects. Southern Forests **77**:41-50.

Harwood, C. E., H. T. Ha, H. Q. Tran, P. A. Butcher, and E. R. Williams. 2004. The effect of inbreeding on early growth of Acacia mangium in Vietnam. Silvae Genetica **53**:65-69.

Kha, L. D., C. E. Harwood, N. D. Kien, B. S. Baltunis, N. D. Hai, and H. H. Thinh. 2012. Growth and wood basic density of acacia hybrid clones at three locations in Vietnam. New Forests **43**:13-29.