PN-AAZ-734

55964

FEASIBILITY STUDY ON TISSUE CULTURE FOR MULTIPURPOSE FOREST TREE SPECIES

by

Dr. Paiboolya Gavinlertvatana
Dr. A. Colin Matheson
Mdm Eng Peng Sim

WINROCK INTERNATIONAL-F/FRED
P.O. Box 1038
Kasetsart Post Office
Bangkok 10903
THAILAND

ACKNOWLEDGEMENT

We would like to express our sincere thanks and appreciation to Winrock International, Bangkok, Thailand for their financial support; to Plantek International (Pte) Ltd, Singapore for allowing Mdm Eng Peng Sim and Dr Paiboolya Gavinlervatana to participate in the project; and to CSIRO, Australia for permitting Dr A C Matheson to join the study team.

Our special thanks to Mr Ken MacDicken for his constant encouragement and support during the study, and to Dr Charles B Mehl and Dr Lee Medema for their comments on the report. We would also like to thank Mr Brian Gambrill and Dr Joseph Kueh for reviewing the manuscript and to Ms Foo Lee Meng and Ms Moh Hoi Cheng for their secretarial assistance.

Last but not least we would like to express our deep appreciation to all the people whom we have interviewed. The support, information and kind co-operation from all of you have contributed to the successful completion of this report.

Dr Paiboolya Gavinlertvatana
Dr A C Matheson
Mdm Eng Peng Sim

20 July 1987

CONTENTS

	Page
ABBREVIATIONS	(i)
LIST OF FIGURES	(ii)
I INTRODUCTION	1
II PRESENT STATUS OF TISSUE CULTURE AND GENETIC RESOURCES OF F/FRED PRIORITY SPECIES	4
1. *Leucaena leucocephala*	4
2. *Acacia mangium*	7
3. *Acacia auriculiformis*	8
4. *Acacia nilotica*	10
5. *Dalbergia sissoo*	11
6. *Eucalyptus camaldulensis*	12
7. *Melia azedarach*	13
8. *Azadirachta indica*	14
III PROSPECTS FOR TISSUE CULTURE AND GENETIC IMPROVEMENT	16
1. Theorectical advantages of tissue culture over other propagation techniques.	16
2. General advantages in the use of tissue culture over seed propagation.	23
3. Program for tissue culture and genetic improvement.	24
3.1 *Leucaena leucocephala* (Lam) de Wit.	24
3.1.1 Integrated genetic improvement/tissue culture.	25
3.1.2 Somatic cell hybridization, somaclonal variant selection and genetic engineering.	29

Page

3.2 Acacia mangium 35

3.2.1 Heart-rot pathology and tissue culture selection for resistance. 35

3.2.2 Seed-based breeding program. 38

3.2.3 Tissue culture micro-propagation. 38

3.3 Acacia auriculiformis 40

3.3.1 Tissue culture micro-propagation of Acacia auriculiformis. 41

3.3.2 Tissue culture micro-propagation of hybrid: A. mangium x A. auriculiformis. 41

3.4 Eucalyptus camaldulensis 43

3.4.1 Tissue culture micro-propagation and mass production by bioreactor. 43

3.4.2 Selection of specific superior traits through tissue culture. 43

3.5 Dalbergia sissoo 47

3.6 Melia azedarach, Azadirachta indica and Acacia nilotica 47

IV RECOMMENDATION FOR RESEARCH AND DEVELOPMENT PROGRAM 48

1. Leucaena leucocephala 48

2. Acacia mangium 48

3. Acacia auriculiformis 49

4. Eucalyptus camaldulensis 49

5. Dalbergia sissoo 49

6. Melia azedarach , Azadirachta indica and Acacia nilotica 50

			Page
V	SUMMARY		51
VI	APPENDICES		
	Appendix 1	Literature review: tissue culture	53
	Appendix 2	References	64
	Appendix 3	Itinerary and useful addresses	75

ABBREVIATIONS

ACIAR	=	Australian Centre for International Agricultural Research
BIOTROP	=	South East Asia Regional Center for Tropical Biology
BOSTID	=	Board on Science and Technology for International Development
CSIRO	=	Commonwealth Scientific and Industrial Research Organisation
F/FRED	=	Forestry/Fuelwood Research and Development Project
FRIM	=	Forest Research Institute of Malaysia
FORI	=	Forest Research Institute
KU	=	Kasetsart University
MPTS	=	Multipurpose Tree Species
MSBP	=	Manila Seeding Bank of Philippines
PNG	=	Papua New Guinea
RFD	=	Royal Forest Department
RRIM	=	Rubber Research Institute of Malaysia
UP	=	University of Philippines
UPM	=	University Pertanian of Malaysia

LIST OF FIGURES

		Page
Figure 1	Genetic progress depends on heritability	19
Figure 2	Comparison of gains between seed orchard option, conventional clonal forestry and tissue culture (adapted from Matheson and Lindgren, 1985)	21
Figure 3	Stylised breeding and tissue culture program for *Leucaena leucocephala*	27
Figure 4	Tissue culture program for *Leucaena leucocephala*: Multiplication of psyllid resistant strains	28
Figure 5	Somatic cell hybridization and hybrid selection of psyllid resistant strains of *Leucaena leucocephala*	30
Figure 6	Somaclonal variant selection for psyllid resistant strains of *Leucaena leucocephala*	31
Figure 7	Diagrammatic presentation of RFLP technology for psyllid resistance	33,34
Figure 8	Tissue culture selection for heart-rot disease resistance of *A. mangium*	37
Figure 9	Selection and tissue culture micropropagation of superior biotypes of *A. mangium*	39
Figure 10	Tissue culture micropropagation of *A. auriculiformis* superior biotypes	42
Figure 11	Tissue culture micropropagation and mass production by bioreactor	45
Figure 12	*Eucalyptus camaldulensis*: selection of specific biotype(s)	46

I INTRODUCTION

The tropical forests, a large area covering about 1935 million ha (FAO, 1980), provide about 1,400 million m^3 of wood annually. Of this about 1,200 million m^3 (86%)is used for fuel. Fuelwood provides 63 percent of the total energy consumption of developing African countries, 17 percent for the countries in Asia and 16 percent for Latin America.

Wood is an important source of energy for the rural communities. Increased fuelwood demand with respect to population growth in the developing rural countries has increased deforestation. Altough plantation establishment in the tropical countries has been accelerated, this equals to only one-tenth of the area deforested every year.

Winrock International, based in Washington, D.C., has established a significant program on the promotion and co-ordination of research and development of multipurpose tree species. This program will boost plantation development and provide information on proper silviculture, management and land use efficiency. Winrock International utilizes new technologies, e.g. computerized data-base and biotechnology in the development program.

Over the past few decades tissue culture, a biotechnology, has rapidly evolved into a major research tool in biology, medicine and forestry. It has reached a level where it can be adapted to large scale industrial utilization. However utilization of tissue culture in forestry, unlike agriculture, is still in its early developmental stage. Reports on the use of tissue culture for forest trees are slowly increasing but further development and research on a concerted effort need to be explored.

TERMS OF REFERENCES AND OBJECTIVES

This report is a feasibility study on the tissue culture of multipurpose forest tree species. It covers the present status of tissue culture of F/FRED priority species, prospects of tissue culture in research and development and offers specific recommendations for the use of tissue culture. Other important and at times viable aspects such as breeding programs, provenance trials etc. are generally not touched upon except when they tie in with the tissue culture programs.

Methodology includes literature review; desk research; field interviews of specialists in Malaysia, Thailand, Indonesia and Philippines, and analysis and draft report preparation. Draft report to be reviewed by 2-3 scientists prior to the final report. Specific recommendations are made on research projects for specific species.

Field interviews were conducted between May 4 and May 18, 1987. Analysis and draft report was prepared at Plantek International, Singapore between May 18 and May 22, 1987. Dr Paiboolya Gavinlervatana, Plantek International, served as tissue culture specialist and team leader; Dr Colin Matheson, CSIRO, Australia, served as forest geneticist specialist; Mdm Eng Peng Sim, Plantek International, Singapore, served as tissue culture scientist.

LIMITATION OF THE REPORT

Due to time and travel constraints (4 countries were covered over a period of 15 days), only selected numbers of persons and laboratories were visited. This report is, therefore, unable to cover all aspects and details. However, we endeavour to present the real situation of tissue culture of F/FRED priority species from data gathered from literature and from our interviews.

As for the research program we focus our recommendation on the use of tissue culture for the improvement of F/FRED priority species.

II PRESENT STATUS OF TISSUE CULTURE AND GENETIC RESOURCES OF F/FRED PRIORITY SPECIES

1. LEUCAENA LEUCOCEPHALA

Leucaena leucocephala is a native of central America. 'Giant' Salvador strains came from Southern Mexico & Guatemala. Breeding system of Leucaena is rather different from the other MPTS. According to Brewbaker (1982), it is predominantly a self-pollinating species with only about 2% outcrossing. This means that 'normal' tree-breeding techniques such as seed orchards are not appropriate. Thus genetic improvement is more difficult. Controlled pollination is essential to create variation within self-pollinated families and to obtain hybrid vigour (heterosis) as they are normally highly homozygous. Genetic improvement must proceed by crossing desirable trees. F_1 progeny will probably be relatively uniform but will not be true breeding as the next generation will segregate. In this segregating array there should be trees which are better than either grandparent and should be self-pollinated for many generations (with intense selection) to obtain true breeding lines. Intermediate gains can be harvested by vegetatively propagating suitable trees at each generation (perhaps by micropropagation).

Leucaena leucocephala is planted most extensively in the Philippines where it is used for firewood and fodder. In the Philippines it is still the primary multipurpose tree species (MPTS) (Esteban). Before psyllid attack became a serious problem, there was a very lucrative market for leaf-meal made from Leucaena leaves fetching around 12000 pesos/yr/ha(Taguda). As harvesting begins 6 months after planting, financial return is fast. The psyllid attack devastated this market with one company (Manila Seedling

Bank Foundation, MSBF) reportedly losing 7 million pesos through this problem alone. A substitute, with almost comparable growth rate, is L. diversifolia. However, one estimate of the fibre content (16%) suggests that its acceptability as a fodder replacement is in doubt. (Taguda - cf L. leucocephala contains around 2-3% fibre). The largest plantations are owned by the National Electricity Administration (c 12000ha) and Manila Seedling Bank Foundation (2000ha). According to MSBF, the growers prefer to plant L. diversifola rather than to test L. leucocephala psyllid-resistant strains because the former is known to be resistant to psyllid.

Even though there is this large investment in Leucaena, the research into finding and/or testing psyllid-resistant strains seems rudimentary. There is an inter-agency psyllid research program with 5-6 projects (including testing resistant seed) but some obvious initial steps (such as collecting seeds from the trees surviving after psyllid attack, and therefore presumably resistant trees) have not been taken.

In Indonesia, Leucaena is planted mainly by agriculturists rather than by foresters. It is not regarded as an industrial species. It is used for interplanting except in Lampung where there is a 1000ha plantation used as firewood source for a steel plant. The psyllid has destroyed millions of trees in Timor, E.Java, Bali etc. and Indonesian scientists are currently testing resistant strains.

Leucaena leucocephala is a promising species for Thailand, but the psyllid problem is spreading there too (RFD, 1987).

Genetic resources: fast-growing strains almost exclusively obtained from Prof. J.L. Brewbaker in Hawaii. There are also putative psyllid-resistant strains available (eg. K636, K156 and K748) which include a hybrid of *L. leucocephala* x *L. diversifolia*. Rangewide provenance trials of *L. leucocephala* do not seem to have been carried out in the region.

Selection criteria: BOSTID (1984) identifies these as having improved growth rate and resistance to acid soils. Psyllid-resistance (or toletance) is an obvious additional candidate (RFD, 1987).

Tissue culture: At present, testing of available seed-based strains of psyllid-resistant *L. leucocephala* and *L. leucocephala* x *diversifolia* is necessary. Mass-propagation of promising trees at each generation of the program is desirable. Progress at each generation should be monitored using tissue culture to preserve intermediate generation selections.

No one that we interviewed is presently working on tissue culture of this species. According to literature, much work has been done on the tissue culture of seedling and mature trees. Successes from various laboratories on tissue culture of *Leucaena leucocephala* has demonstrated this to be a viable technique for mass propagation and improvement (Goyal, Y. et. al, 1985 ; Datta, S.K. and K. Datta, 1984). Tissue culture of *Leucaena diversifola* is also successful (Venketeswarn, S. *et. al*, 1984). Plant regeneration for callus has been obtained in both species, but protoplast culture is not yet successful.

ACACIAS

Acacias are generally assumed to be predominantly outcrossing but hard data are rare (Sedgley-1986). Pollination is by bees, other insects and birds. This suggests that Acacias are amenable to breeding through traditional means by seed orchards.

2. ACACIA MANGIUM

A. mangium is native to North Queensland, Australia; Southwest Papua New Guinea (PNG); Irian Jaya and Moluccas Indonesia. The largest plantations are in Sabah, Malaysia as it is the principal species in a 200,000 ha reforestation scheme (BOSTID, 1983). There is a breeding program for the species which supplies seeds at a high price. Mangium is also being planted on a large scale in Peninsular Malaysia as a stop-gap measure before indigenous plantations come on stream. It is also planted in South Sumatra and South Kalimantan, Indonesia and in Mindanao, Philippines where there is no dry season. Its growth is very much slower in dry areas.

Genetic resources: The first introductions to Sabah were via a single half-sib family but by the 3rd generation they were growing only half as fast as the 1st generation trees (Sim, 1984). Breeding and introduction program has however improved the performance. There are provenance trials in many countries now. Analysis of the data is still in progress.

Selection criteria: Selection for resistance to heart-rot is necessary as A. mangium seems susceptible to (BOSTID, 1983). There is some pathology work going on in Malaysia (Lee 1986), but the causal organism is unknown. It seems to spread from pruned branches to the heartwood and affects up to 30% of 3-4 year old trees in Sabah (Ng). It has been found in Peninsular Malaysia, Indonesia but not in Philippines nor Thailand perhaps because the weather is drier or pruning is not carried out.

Branch form is also a selection criteria plus, of course, increased growth.

Tissue culture: Report on tissue culture of Acacia mangium has been minimal (Rajadurai, D.D., 1987). There has been very good progress in those laboratories that we visited including UPM, Malaysia; RFD, Thailand; and BIOTROP, Indonesia. Tissue culture of mature trees has been successful as well as that of seedlings. This provide a viable technique for mass propagation of superior biotype(s) and further improvement by in vitro selection for heart-rot disease resistance.

A. mangium hybridises with A. auriculiformis and the hybrid seems to combine the high density of A. auriculiformis with the better stem form of A. mangium. Work is beginning at UPM, Malaysia on the phenology of the two species and the hybrid.

3. ACACIA AURICULIFORMIS

Natural range is in north Queensland, Northern Territory and Northern Western Australia and PNG. It is widely planted as a street tree in Indonesia, Thailand and Sabah. The

genetic base of the Sabah introductions is thought to be narrow (Sim, 1986) but has since been broadened with new introductions. The stem form of the species is generally poor. Even in relatively close spacings the stems are crooked, particularly in Indonesia and Malaysia. However, there exist tall and straight trees at Sai Thong in Thailand (see photo). It is said to be widely adaptable and will grow on drier sites than A. mangium.

Genetic resources: These are generally as poorly understood as those of A. mangium. The origins of early introductions to the region of interest are unknown. New provenance collections for F/FRED from throughout its range are timely. These collections were from PNG (3 provenances of about 50 parent trees each, plus bulk collections) Cape York (10 provenances of 10+ trees each), Northern Territory (10 provenances of about 10 trees each), and from the Kimberley Ranges in Western Australia. There are large and obvious differences in ASEAN provenance trials so far and, in particular, local provenances perform much worse than those from Australia. We were told of large differences within provenances, but it is unclear whether these are due to different mother trees or outstanding individuals within families.

Selection criteria: Clearly, improvement in stem form would be the major selection criteria. The Sai Thong area seems to have produced some outstanding trees in this respect (see picture 1, page 40), but the seed origin is unclear. Although there are some poor trees surounding the desirable individuals at Sai Thong, it is possible that site factors may play a part. The genetics of this improved stem form need to be studied. Again, increased growth rate is an important criteria.

Tissue culture: There is no literature on tissue culture of A. auriculiformis. Progress has been made at RFD, Thailand, thus showing a potential for tissue culture of A. auriculiformis.

If the outstanding individuals reported from Sai Thong are very few, or the improved stem form is due to non-additive genetic effects (refer page 16 for theoretical explanation), then tissue culture of well-formed trees is a prospect. However, well-formed trees were not reported from anywhere else and it may be an effect of sandy, well-drained soils rather than a genetic effect. The hybrid between A. mangium and A. auriculiformis deserves more research. Sim (1986) reported that the hybrid is more vigorous and has better form than A. auriculiformis with lighter branching. A selection program beginning with F_2 hybrids should be put into effect and desirable individuals in each generation could be propagated by tissue culture.

4. ACACIA NILOTICA

A. nilotica is native to the Sind, Punjab and Deccan areas of the Indian sub-continent (subsp. indica), and to Africa (ssp. nilotica). It is popular throughout India and Pakistan for firewood, fodder and tannin (BOSTID,1980). This species is little known in the ASEAN region. It has been introduced to Indonesia where it is very aggressive and is now regarded as a pest. It was initially used as protection species around crops, but has frequently invaded the crop areas, taking over large areas (Komar). The Indonesians would rather eradicate it than introduce it further. Note should be taken of the warning on page ix of BOSTID (1980) about the introduction of potentially invasive plants, and on page 99 concerning its introduction only to arid areas with a critical shortage of firewood.

Genetic resources : Little seems to be known about the sources of introduction of A. nilotica to the region. Little interest was expressed in it as a species.

Tissue culture: There was no report found on tissue culture of this species. As it is a profuse seeder, potentially invasive and with seemingly no pathological problems in the region, there is little prospect for tissue culture.

5. DALBERGIA SISSOO (SISSOO)

Sissoo is native to India and now grows widely throughout the sub-continent, and in dry parts of Africa. It is also part of a reforestation program in Nepal (White, 1986). Its timber is used for firewood, cart wheels and axles, ploughs etc. and its leaves are used for fodder.

Genetic Resources: Nothing could be determined concerning the genetic resources of this species. Few trees were seen and little interest was expressed in it except in Indonesia where it and another species D. latifolia is very valuable. There is some confusion about whether D. sissoo or D. latifolia is planted at Lampung by the Forest State Corporation (Fakuara). There are only small-scale plantings in Thailand.

Tissue culture: Tissue culture of Dalbergia sissoo has been reported (Datta, S.K. et. al, 1983). Successful tissue culture of mature trees suggests a viable program on mass propagation and improvement. BIOTROP, Indonesia has been working on Dalbergia latifolia with limited success. Seeds of sissoo were said to be in short supply in Indonesia, but there was again confusion between it and D. latifolia.

6. EUCALYPTUS CAMALDULENSIS (RIVER RED GUM)

E. camaldulensis is native throughout Australia where it typically grows along rivers and creeks. Its timber is used for general construction and firewood in Australia and elsewhere. It is very widely planted throughout the world (about 500,000 ha - BOSTID 1980).

Genetic Resources: Eucalypts are generally outbreeders although about one-third of seeds collected from the wild are derived from self-pollination. A single generation of true outcrossing in a seed orchard or seed production area would probably increase average growth considerably. Selection of the correct provenance is critical for this species e.g. varieties 'Petford', 'Katherine' and a few others grow best in the humid tropics. There are provenance trials of the species in Thailand. E. deglupta is preferred for its better form in the Philippines but there is considerable interest nevertheless. There was some success recently with cuttings of E. camaldulensis in Thailand, but the genetic origin of the material was not given. E. camaldulensis does not grow well in Peninsular Malaysia.

Tissue culture: Tissue culture of E. camaldulensis has been reported successful (Hartney, V.J, 1982 ; Gupta, P.K., et. al. 1983). Plantek International (Singapore), RFD (Thailand) and Halos (Philippines) have also successfully obtained tissue culture of E. camaldulensis. Although Aracruz, Brazil has shown advantages of clonal planting by using cuttings of E. grandis, cuttings of E. camaldulensis are difficult to produce. Tissue culture seems to be a good tool for mass propagation of superior trees and Eucalypts improvement.

As with other species, breeding programs for E. camaldulensis are in the very early stages and then only in Thailand. There are good prospects for large-scale plantings in Thailand and the Philippines. Correct selection of provenance and identification of desirable individual trees is necessary before commercial-scale tissue culture could be contemplated. No particular seeding problems were identified. In Australia, tissue culture for multiplication of salt-tolerant clones is a commercial proposition (Hartney, 1986). If salt-tolerance is a problem in ASEAN countries, this would be a useful source of collaboration. But in the absence of such specific problems the immediate use for tissue culture has not been established.

7. MELIA AZEDARACH (WHITE CEDAR)

This species probably originated in Baluchistan and Kashmir but has been cultivated widely in the Middle East and India (BOSTID 1983b) and the var. australasica is native to the eastern coast of North Queensland (Tumbull, 1986), var. japonica grows in South China (Suree). Selections grown in Argentina for commercial fuelwood production are called var. "gigantica" (Suree). There is some confusion over the relationship between Melia azedarach and Azadirachta indica. They are described as synonyms by BOSTID (1980) but close relatives by BOSTID (1983b). Dr Suree said that they were the same and Azadirachta was called Melia in former times. Dr Rao said that they were different and that Azadirachta is called "neem" and Melia "little neem". Melia seeds are used for nematode control (Rao) and to kill fish in Indonesia (Fakuara).

Genetic resources: There is a private breeding program in Thailand (Suree) involving some 50 selected trees (of unknown provenance), 3 seed production areas and studies involving genetic variation. Seeds collected from various provenances are on trial at Ratchaburi, Thailand.

Selection criteria: The wood of this species is soft and brittle (BOSTID, 1983b) and may respond to selection to improve it. Apart from its use as veneer and fuelwood species in Thailand, its main use seems to be the insecticidal properties of its leaves and seeds. There may be some prospect of enhancing this through selection and breeding.

Tissue culture: Tissue culture of this species was limited but with some success (Vilash, V.,1983). There was not much interest expressed to us. This species (like Azadirachta) is a profuse seeder and, in the absence of strong selection criteria or a large scale planting program, prospects for tissue culture would seem limited.

8. AZADIRACHTA INDICA (NEEM)

Neem is said to be potentially one of the most valuable of all arid-zone trees (BOSTID, 1980). It is native to dry forests in India, Pakistan, Sri Lanka, Thailand, Burma, Malaysia and Indonesia. It is used widely in Africa for fuelwood and the timber is excellent for furniture and construction. In addition, the seeds are used to make neem seed cake (a treatment for nematodes) and neem oil is used as lamp fuel and as systemic insecticide (BOSTID, 1980; Rao). A chemical derived from neem was reportedly better for malaria prophylactic than quinine (Suree). It is growth in alternate rows with Eucalyptus camaldulensis at Ratchaburi, Thailand where seedlings are raised for local distribution.

Genetic resources: In spite of having many valuable uses for its products, there seems little effort on a breeding program, or on exploration of its genetic resources. It is included in the ACIAR provenance trials at Ratchaburi, Sakarat and at Chanthaburi in Thailand, but only the local provenance is used at each place.

Selection criteria: As in *Melia azedarach*, neem has chemical properties which could well be enhanced by selection and breeding. Increased growth would also seem a suitable criterion for planting in alternate rows with *E. camaldulensis* as it is less than 1/3 as tall.

Tissue culture: Tissue culture of this species is limited (Schulz, F.A, 1983). This species is a prolific seeder and this would appear to be the preferred propagation method.

III PROSPECTS FOR TISSUE CULTURE AND GENETIC IMPROVEMENT

1. THEORETICAL ADVANTAGES OF TISSUE CULTURE OVER OTHER PROPAGATION TECHNIQUES

It is generally assumed that any form of clonal propagation for a quantitative character will lead to greater gain than seed-based propagation (McKeand 1981). This assumption is based on the observation that the few outstanding individuals found in a seed-based planting could be reproduced identically by vegetative propagation. For this to be realised, the superiority of the outstanding individuals must be genetically based and the vegetative process must be able to duplicate the superiority without alteration. This has been proven to be true in *Eucalyptus* sp. in Brazil and fruit trees in Asia and elsewhere.

To predict the performance of trees when cloned and to compare this with predicted seedling performance, several genetic parameters are required. The gains made from seed propagation depend on the additive genetic variance (due to average effects of genes) whereas those of clonal propagation depend on total genetic variance (additive plus non-additive effects) (see Figure 1). Non-additive variance is due to particular combinations of genes. For example, human eye colour is controlled by a pair of genes, one for blue and one for brown with each person carrying two such genes. If the person is a heterozygote, carrying a blue gene and a brown gene the two do not add together, but act as a pair to produce brown eyes just as if the person carries two brown genes. Such an effect is called 'dominance' and is the major component of non-additive variance.

The greater gains to be made from clonal forestry (including tissue culture) over seed-based forestry are frequently assumed to be due to the non-additive variance which can be used by clonal forestry but not by seed-based forestry.

Although non-additive variance is usually smaller than additive variance, another major component of clonal forestry's advantage is time (Matheson and Lindgren 1985). This means that clonal forestry's principal advantage is that the gains made in a tree breeding program may be utilised in plantations sooner and that selection for plantation can be delayed to take advantage of better selection from the breeding program. Matheson and Lindgren (1985) considered two kinds of breeding program, one for a northern European conifer taking about 25 years to complete a cycle of breeding, the other for a faster-growing temperate pine taking only 15 years to complete a breeding cycle. In the case of tropical species modification to the arguments is required.

Table 1 compares time and genetic advantages in using clonal plantations with seed-orchard based plantations. It is assumed that breeding cycles take 6 years, seed orchards take about 4 years and clones take about 3 years to be fully productive. Units in Table 1 and in the Figures 2A & 2B are based on k V_a, where k is a constant factor describing the correlation between the characters assessed in field trials and the end-use characters and V_a is the additive genetic variance.

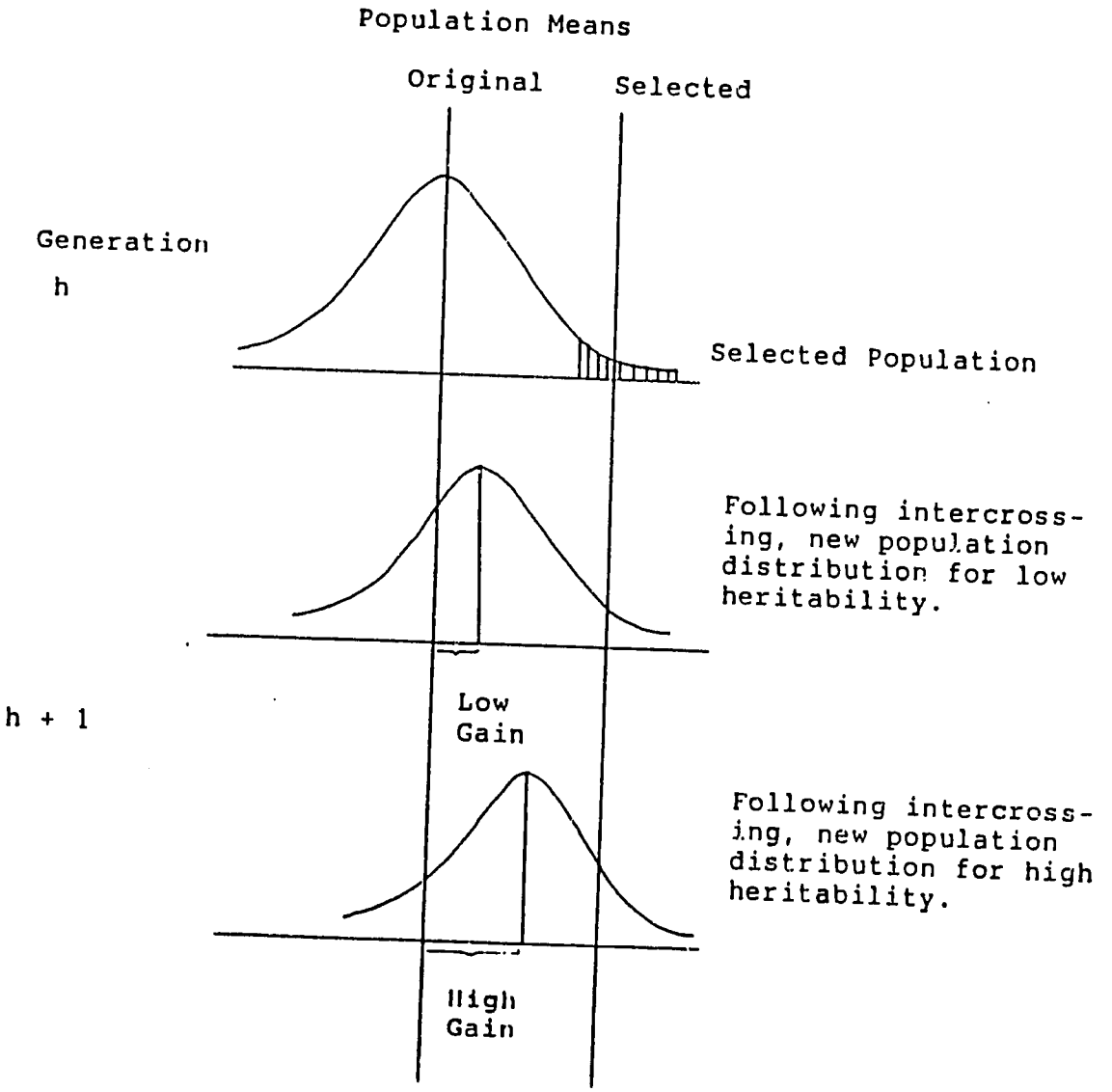
Table 1: Time and genetic advantages in using clonal plantations over seed-orchard based plantation

	Non-additive variance = 0	Non-additive variance = Additive variance
Time advantage	0.05987	0.04888
Genetic advantage	0.33579	2.06737
Total	0.39565	2.11625

We assumed a time scale for clonal production of 3 years, appropriate for tissue culture but would probably be longer for classical vegetative propagation (if it is possible in these species). The major component of the advantage is the genetic advantage - extra gain through the use of the same genotypes as those assessed in field trials. This genetic component is largely dependent on the amount of non-additive genetic variance in characteristics of interest in the MPTS. As yet, these genetic parameters are unknown.

Figure 1: Genetic progress depends on heritability

For seedling propagation heritability depends on additive portion of genetic variance. For vegetative propagation heritability depends on both additive and non-additive genetic variance.



(After Matheson and Brown, 1983)

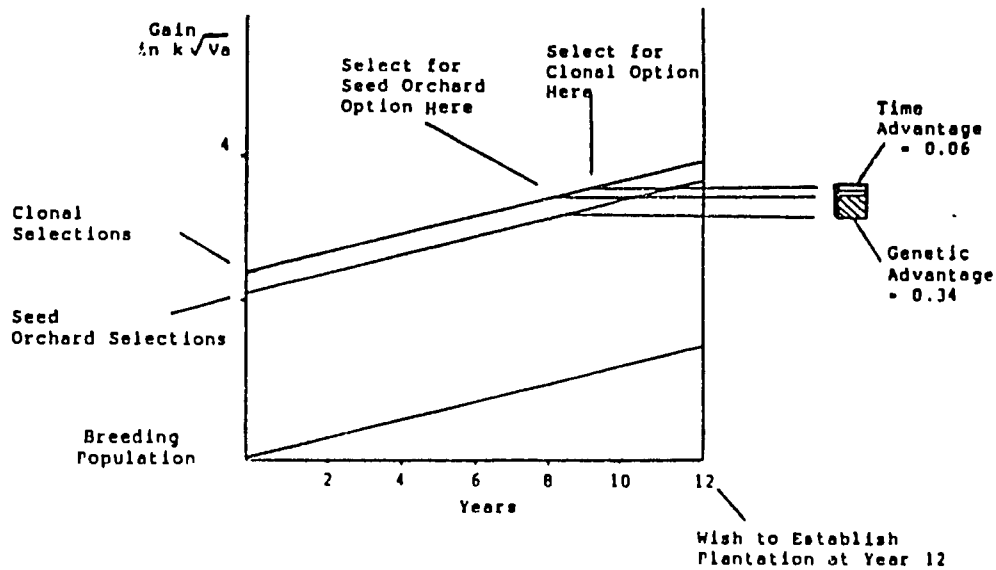
Notes for Figure 1

Heritability is a function of additive, non-additive and environmental variances. It is the proportion of the otal variation attributable to additive variance (or average effects of genes). Repeatability is the proportion of the total variation attributable to genetic (ie. additive plus non-additive) variance. Non-additive variance is caused by the deviation of particular combinations of genes from the average.

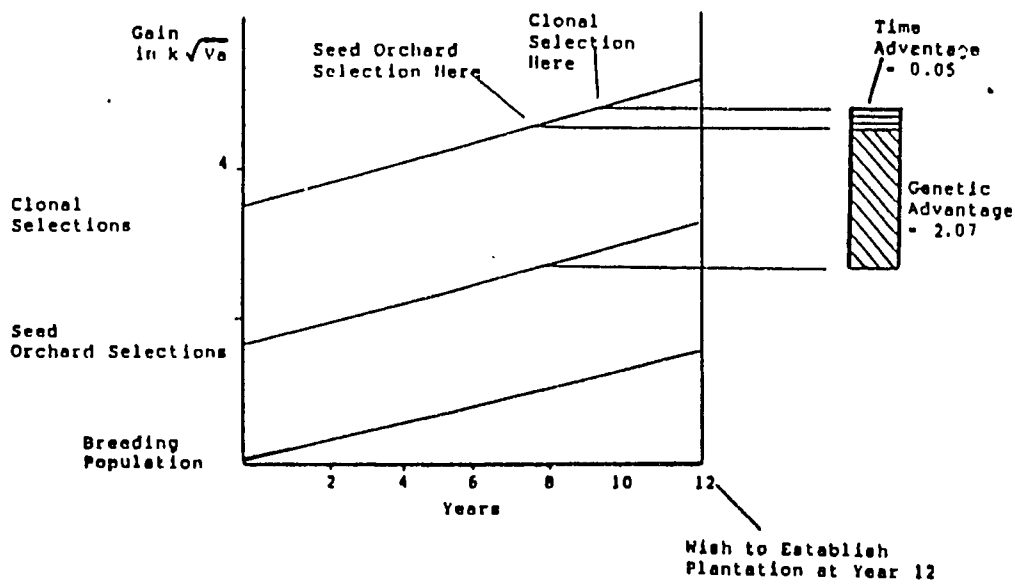
The higher the heritability, the greater the resemblance (in performance) of trees to their selected parents. For low-heritabiltiy traits, progeny tend to resemble the average of the previous generation. For vegetative reproduction, high repeatability means that plantlet (or ramet) performance resembles that of the original plant (ortet). This is particularly applicable to tissue culture. Because the genetics of somaclonal variation is imperfectly understood, uniformity of the products of tissue culture of a clone is not assured without testing.

Figure 2: Comparison of gains between seed orchard option, conventional clonal forestry and tissue culture (adapted from Matheson and Lindgren, 1985)

A: Assume non-additive variance = 0



B: Assume non-additive variance = additive variance



At present there are no estimates of additive and non-additive variance for use as selection criteria in MPTS and hence no basis for making calculations of monetary gains. The units of gain calculated above are for an idealised system according to the formula $K\sqrt{V_a}$. Additive and non-additive variances are best estimated from progeny trials. There are open-pollinated progeny trials of A. mangium and A. auriculiformis in Sabah (Sim 1984, 1987) which could yield appropriate estimates, particularly for growth and form traits, but which must await harvest for the assessment of the genetics of resistance to heart rot.

These calculations on the genetic advantages of tissue culture do not apply to Leucaena leucocephala which is a self-pollinating species (Brewbaker, 1982). In this case, the seed-orchard option is not possible and seed-propagation is through inbred lines. To produce a stable, true-breeding line would take many years of self-pollination, testing and selection. The advantage of tissue culture in this case is that plants with good characteristics which are still heterozygous and therefore not suitable for seed production can be selected, cloned and multiplied to supply uniform planting material to the market.

We presume, with the lack of certain information, that Acacias are all predominantly outcrossing. The only Acacia species so far reported, A. harpophylla, A. mearnsii and A. decurrens (Sedgley, 1986), are all temperate Australian species and perhaps not relevant to the tropical species under consideration. We assume that seed-orchards, already existing for A. mangium and A. auriculiformis, are a suitable method for generating seeds for commercial use.

2. GENERAL ADVANTAGES IN THE USE OF TISSUE CULTURE OVER SEED PROPAGATION

A. Micropropagation. Tissue culture provides a means of mass propagation on a very large scale. Where conventional cuttings are also feasible then large numbers of mother trees occupying large areas are required by this method to supply material for a substantial planting program. In this case, the relative costs of each method need to be assessed before deciding whether to use tissue culture or conventional vegetative propagation method.

If conventional cuttings are diffcult (as for *A. mangium*, Sim 1987), the use of tissue culture would be advantageous for mass propagation. Production of clones for seed orchards may still be best done by conventional means (e.g. marcotting - Sim 1987) as small numbers of plants are required.

If seeds are in short supply, or expensive (e.g. US$1,200/kg for mangium seed from Sabah to the Philippines), micropropagation could be a viable alternative.

B. Cheaper. For very large-scale operations, micropropagation could provide a cheaper source of plants if seeds are scarced and expensive. However, for small to medium-sized operation, seed or conventional cuttings may be cheaper depending upon circumstances.

C. Uniformity. In theory, clonally-propagated plants are more uniform than those produced from seed. This is so for conventional cuttings of *Eucalyptus* in Brazil. However tissue culture of oil palm has give rise to variation and therefore uniformity must not be

taken for granted. Uniform trees in plantation are always easier to manage than non-uniform ones.

D. Quarantine. There is an increasing awareness of seed-borne diseases (FAO, 1987). Quarantine procedures are likely to become stricter in the future. Therefore disease-free material from tissue culture may become the preferred form of exchange of plant material.

E. Specific Problems. Where there are very few trees genetically suitable for use in plantations (e.g. psyllid-resistance hybrids or heart-rot resistant plants), rapid multiplication by tissue culture is desirable. Seed or conventional propagation would be too slow in such cases.

F. Potential for in vitro selection. Where resistance to a pest or pathogen can be ascribed to an identifiable characteristic in culture, then the need for field testing is much reduced. In some cases, resistance to chemical stress can be selected for by including the stress in the growth medium.

G. Potential for genetic engineering. Tissue culture is an essential prerequisite for genetic engineering. If specific traits are required in an organism (perhaps transferring the insecticidal qualities of neem leaves to *Leucaena leucocephala*), it may be possible to do this by genetic engineering.

3. PROGRAM FOR TISSUE CULTURE AND GENETIC IMPROVEMENT

3.1 LEUCAENA LEUCOCEPHALA (LAM) DE WIT

The major problem at present with *L. Leucocephala* is psyllid infestation. Although resistance to acid soils and growth rate are seen as additional selection criteria, these can be screened for in field trials

using selection indices or independent cutting. We concentrate on the psyllid problem here.

We suggest three possible approaches, not mutually exclusive, to provide psyllid-resistant or psyllid-tolerant material in the short, medium and long term. The first is an integrated genetic improvement/micropropagation approach. It will yield new planting material in large quantities in the short, medium and long term, keeping ahead of any genetic changes in host range of the psyllid. The second is almost exclusively a tissue-culture project, involving multiplication of psyllid resistant materials, somatic hybridisation and selection via field trials. The third is again via tissue culture, but also involving genetic engineering for transferring psyllid-resistance genes into desirable strains.

3.1.1 Integrated genetic improvement/tissue culture

This program involves the selection of individual resistant trees as a first step. Seeds of resistant trees are currently in short supply so we believe a micropropagation program is justified for these trees. These and resistant trees selected locally should be field-tested. Once resistant trees have been securely identified, they should be multiplied by tissue culture for distribution to growers. Resistant trees should also be crossed and a breeding program begun so that the degree of resistance may be increased and other characters such as resistance to acid soils and increased growth rate may also be selected for. Because of the breeding system of *L. leucocephala*, the hybrid lines produced in the crossing program will not be true-breeding. Seed should be field-tested at each generation and selected trees multiplied by

tissue culture as well as propagation by seed for further selection. This program is summarised in Figure 3. Tissue culture details are given in Figures 4, 5, 6 and 7.

Breeding and field trials at present have some technical problems. Since tissue culture protocol has been established (Veneketeswan, S. et. al., 1984; Goyal, Y. et. al., 1985 ; Datta, S.K., and Datta, K., 1984), the main technical problem is that the scale-up procedures need to be developed for rapid multiplication. Tissue-culture plantlets must, at this stage, be tested in the field. Tissue-culture plantlets are more expensive than seedlings, but since psyllid-resistant seeds are in short supply, tissue culture is a viable alternative. In view of the importance of the species to several countries in the region (particularly the Philippines and Thailand), the expressed priority for a breeding and selection program (RFD, 1987) and the prospects for success in the short, medium and long term, we believe that this program should be given the highest priority.

Tissue culture micropropagation would require between 10 to 18 months before ramets are ready for outplanting.

Figure 3: Stylised breeding and tissue culture program for *Leucaena leucocephala*

1. Select suitable fast-growing psyllid-resistant and inbred lines. Collect seed from them and from psyllid-sensitive lines for comparison

2. Intercross in all possible combination (= diallele cross)

3. Grow F_1 progeny and estimate additive & non-additive variances.

4. Collect (selfed) seed of F_1 progeny

5. Grow F_2 in progeny trial.

6. Select elite F_2's and micro-propagate for commercial purposes.

7. Collect seed from F_2 elite trees ---→ F_3.

8. Grow F_3 in progeny trial.

9. Select elite F_3's and micropropagate for commercial purposes.

10. Proceed for several generations.

When progeny trials reveal no more segregation, cross the elite trees again and start again from (2)

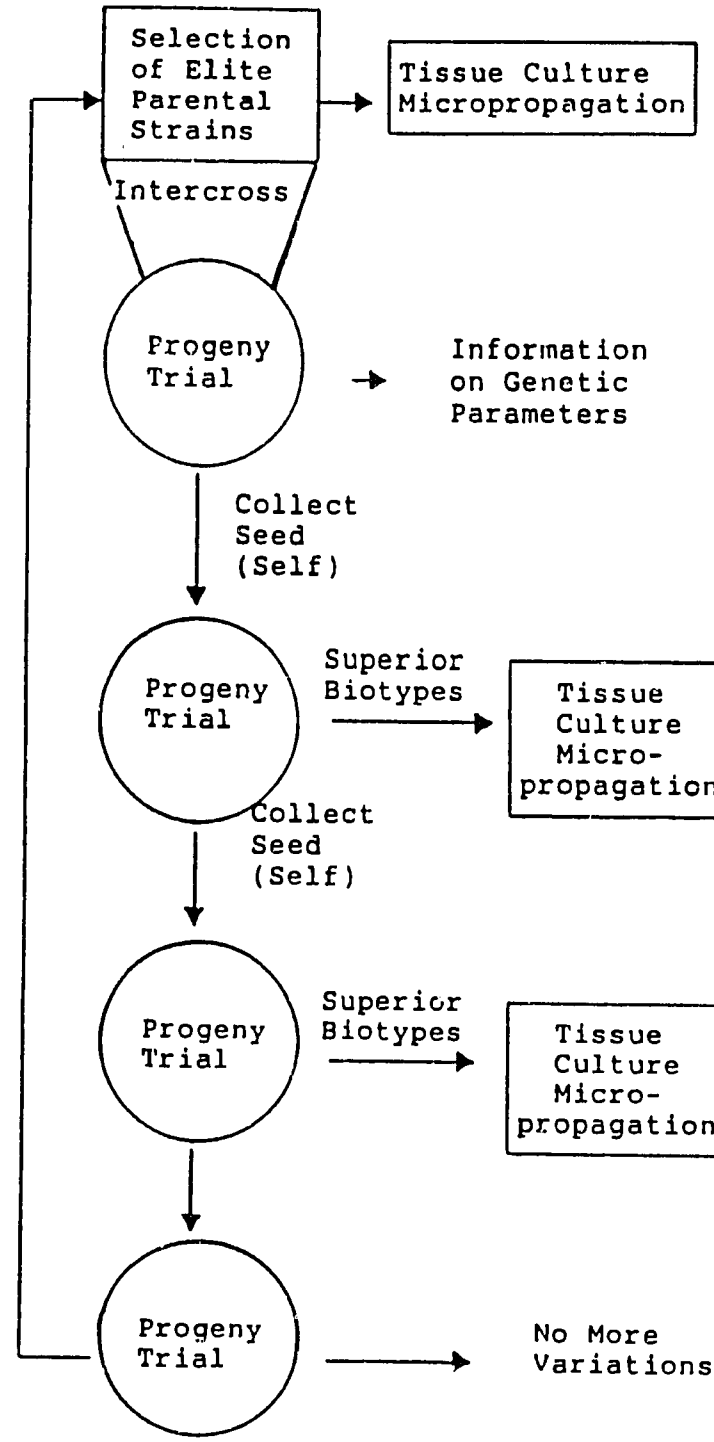
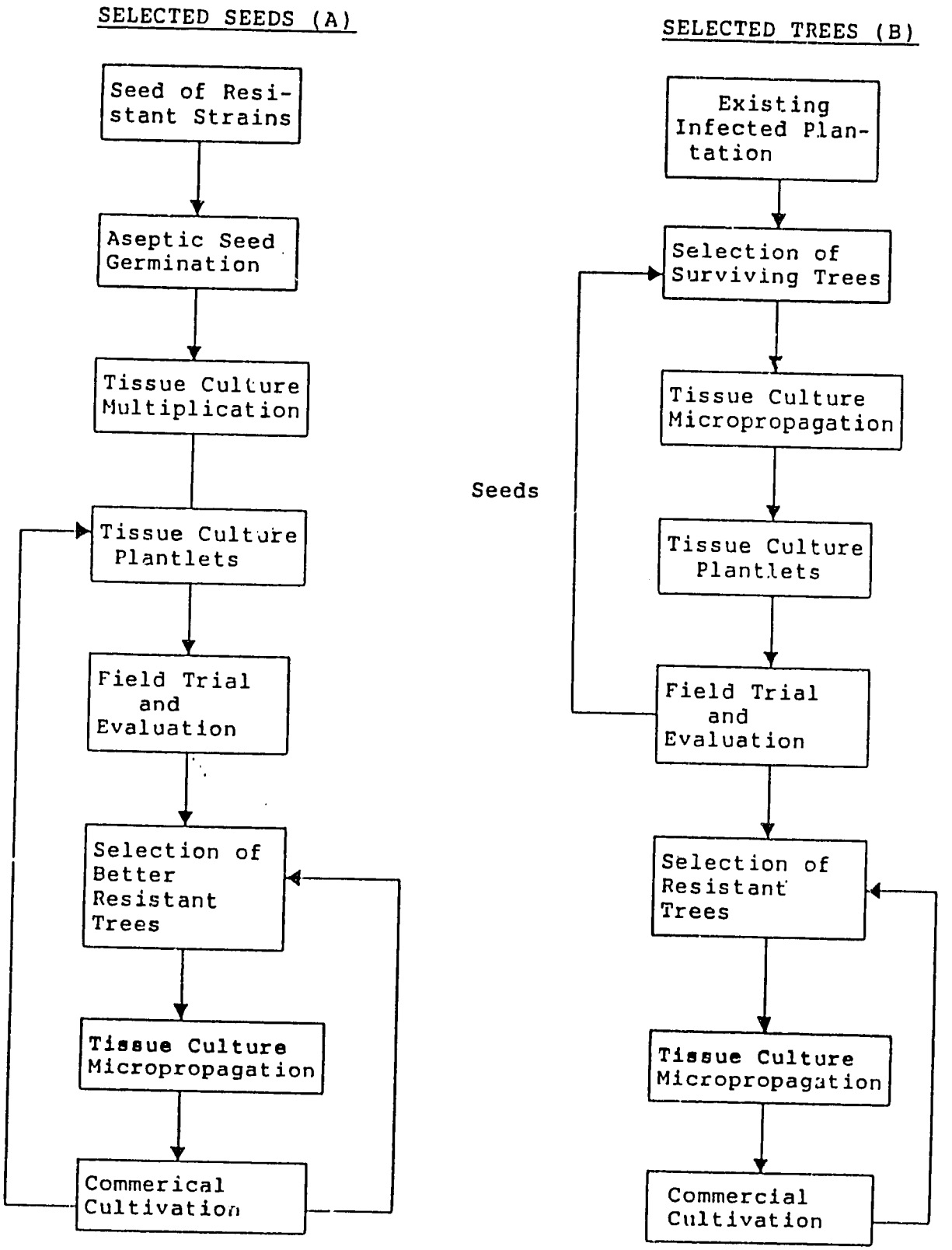


Figure 4: Tissue culture program for Leucaeua leucocephala : multiplication of psyllid resistant strains



3.1.2 Somatic cell hybridization, somaclonal variant selection and genetic engineering

The rapid development of cell culture and molecular biological techniques over the past few years has reached a stage where they could now be applied in agriculture and forestry.

By protoplast fusion between *L. leucocephala* (a susceptible species) and *L diversifola* (a resistant species) (Figure 5), hybrids containing resistant characteristic of *L. diversifola* could be obtained. Undesirable characteristic (i.e. high fiber content) may be present in the hybrids. Critical selection and screening procedures must be carried out. This technique though providing a feasible tool to produce resistant hybrid does not appear to be advantageous over conventional crossing as the two species are sexually compatible. In addition, a long lead time for research, perhaps 5 years, prior to planting out of ramet will be a limiting factor. Successful conventional hybridization between the two species has been reported. Therefore, efforts should concentrate on crossing and field assessment. Selection of desirable trees, then mass propagation by tissue culture must be emphasized. However if pysllid resistance is not nuclear-coded, i.e. cytoplasmic inherited, protoplast fusion would be applicable.

Somaclonal variation arising from tissue culture (Figure 6) allows selection for variants resistance to psyllid. Research program may require 2 - 3 years prior to planting ramet for field assessment.

Figure 5: Somatic cell hybridization and hybrid selection of psyllid resistant strains of Leucaena leucocephala

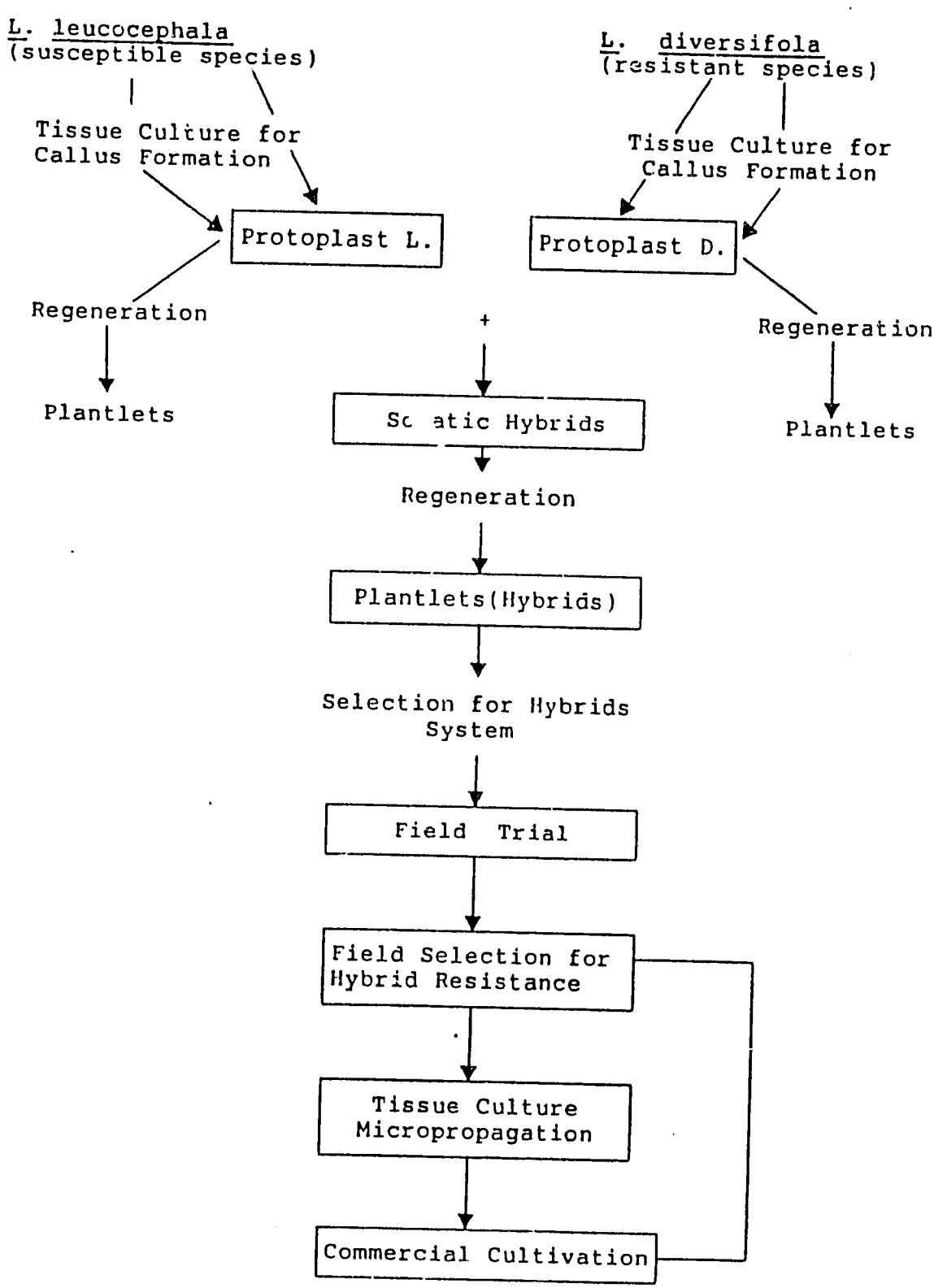
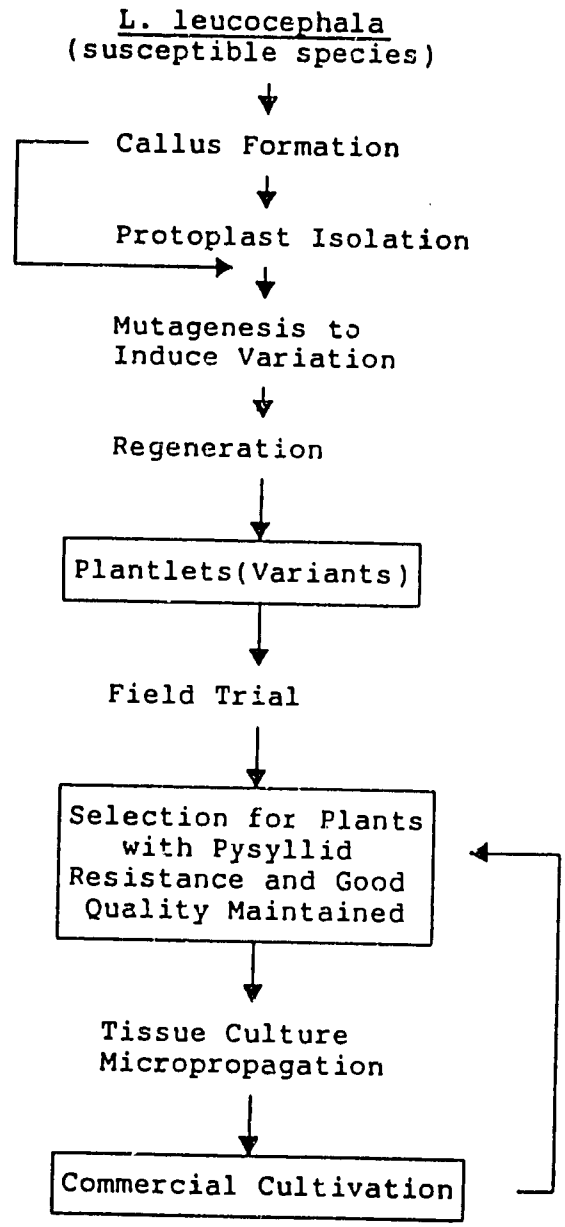


Figure 6 : Somaclonal variant selection for psyllid resistant strains of *Leucaena leucocephala*



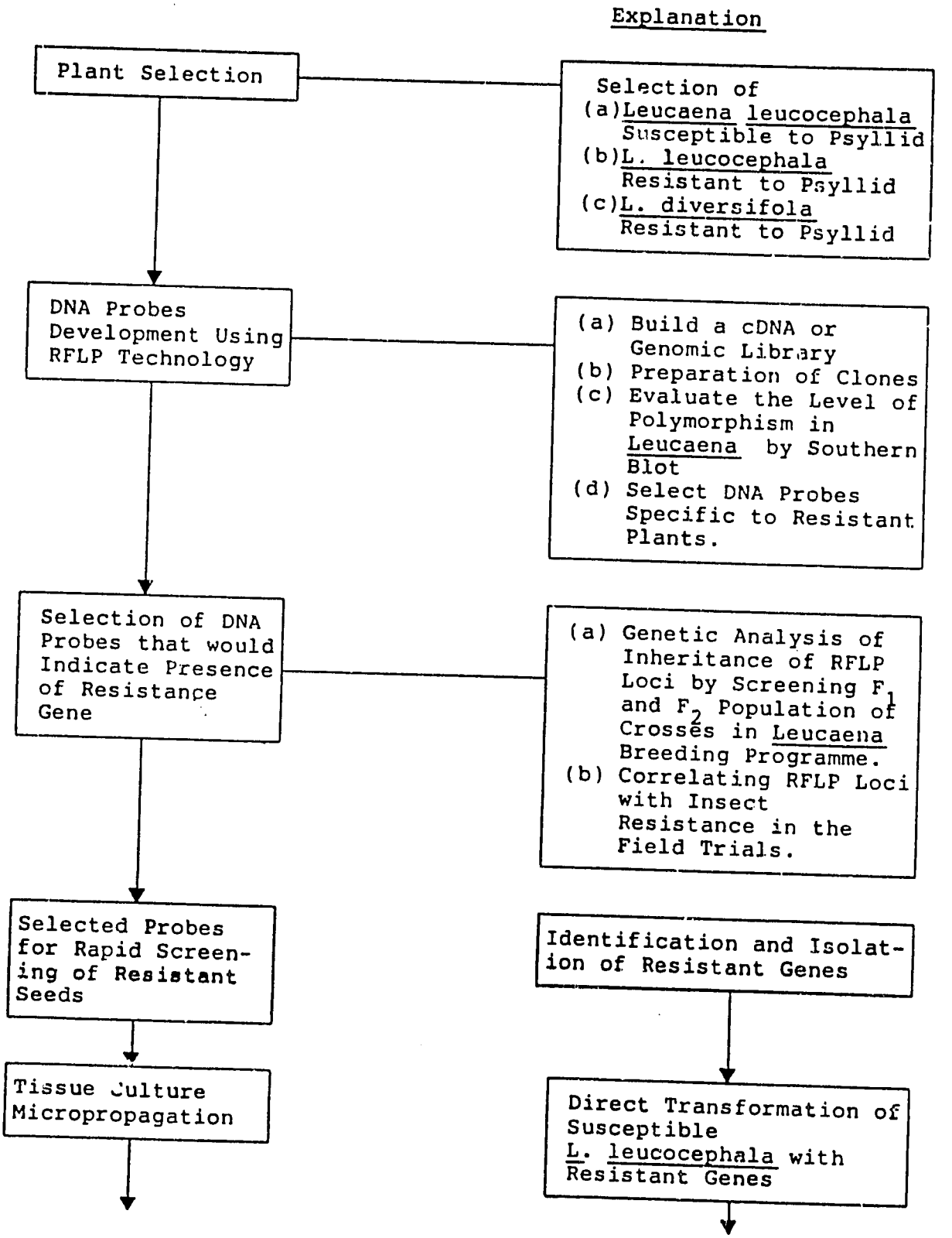
Restriction fragment length polymorphism (RFLP) technology as shown in Figure 7 suggests a long term (at least 5 years before field assessment) but valuable program to encounter psyllid problem.

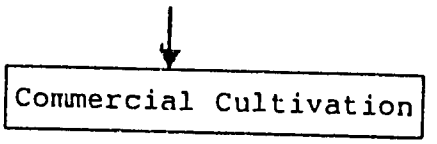
Plant selection is an important step. Leucaena plants already proven to be either susceptible or resistant to psyllid must be used for study. Although RFLP does not require knowledge of the genes involved, selection of probes for an unlinked multigene-coded character would be more difficult. The probes that are developed would allow rapid screening of seeds from crosses. They could eventually be used to isolate the resistant genes which could then be used for direct transformation and to understand the mechanism of insect resistance on the molecular level.

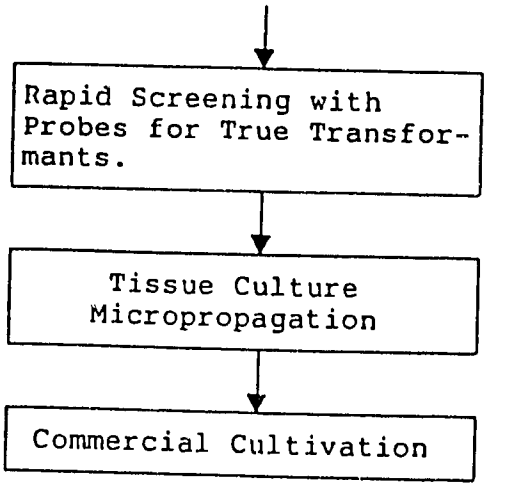
In the process, many other genes would have been cloned and this would indeed be useful for understanding tree genetics and, perhaps more importantly, lead to identification of other genes of desirable characters.

This technique has been utilized at Native Plants Inc., USA, and Plantek International has access to the technology. There is no laboratory in Asia using RFLP technique and this needs to be introduced.

Figure 7: Diagrammatic presentation of RFLP technology for psyllid resistance




Commercial Cultivation


Rapid Screening with Probes for True Transformants.
Tissue Culture Micropropagation
Commercial Cultivation

3.2 ACACIA MANGIUM

Mangium has heavy branching during early growth, is susceptible to heart-rot and is difficult to propagate on a large-scale by cuttings. Superior trees, straight stem with good form and fast growing, have been selected and a seed-orchard is in operation in Sabah. Breeding programs elsewhere are rudimentary and improved seed is expensive and in short supply. We identify three approaches towards improvement required for *A. mangium* in the region.

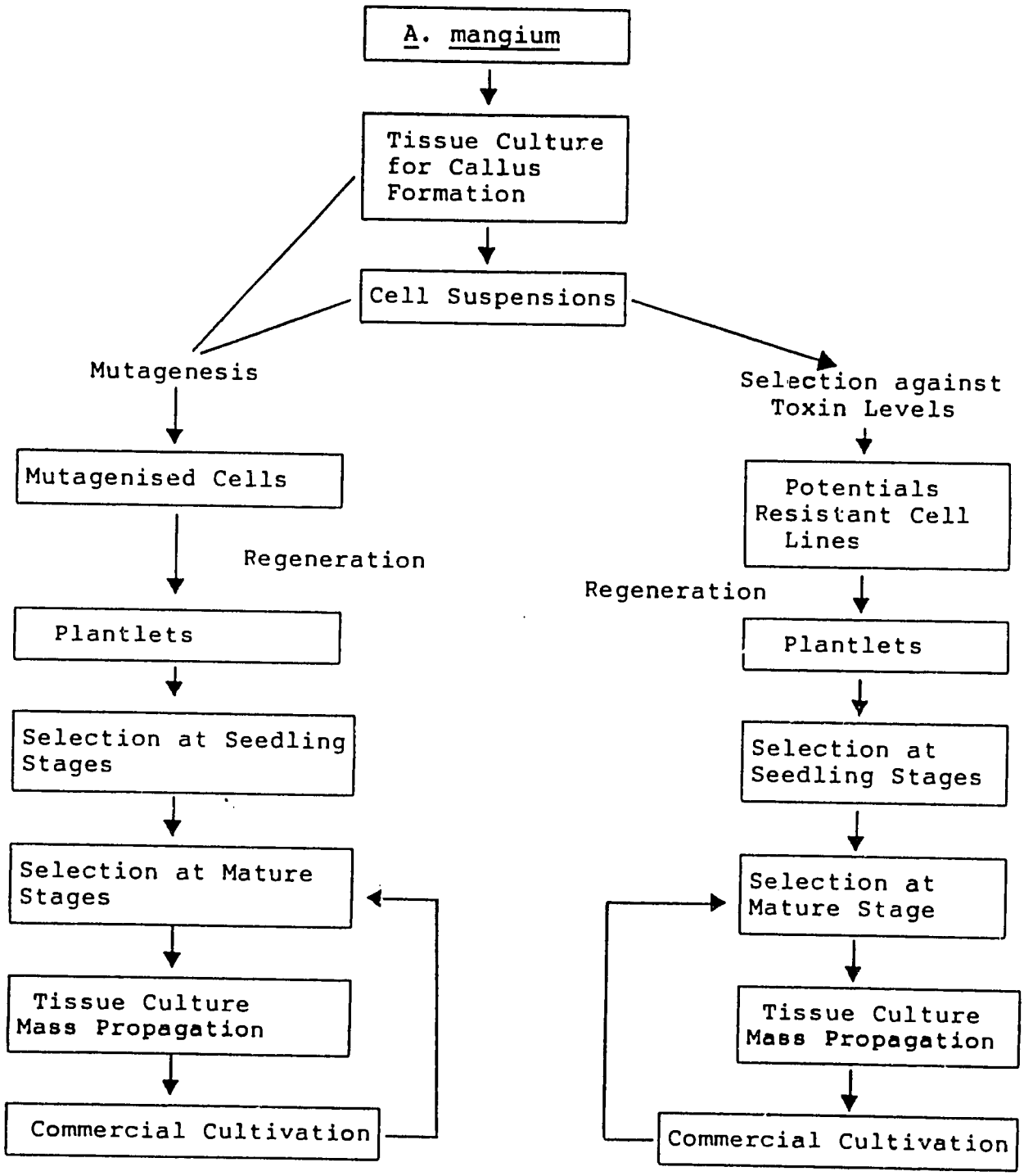
3.2.1 Heart-rot pathology and tissue culture selection for resistance

The biological basis of the heart-rot disease must first be established. This problem currently affects timber quality of about one-third of the trees examined in Malaysia. It is important to identify the causal organism and develop a hazard-reduction procedure-either by silvicultural practice (e.g. altering pruning regimes) or genetical approach (e.g. via a breeding program or biochemical selection using tissue culture as illustrated in Figure 8.) Without a solution to heart-rot, mangium-based paper industry may not be viable.

Tissue culture program consists of mutagenesis and selection pressure against the micro-organism or toxin extract. This technique has been successfully applied to isolate sugarcane resistant to Fiji disease, maize resistant to Southern Corn Leaf blight disease, tomato and tobacco resistant to herbicides, etc. Plantek intends to use this technique to select pepper plants for resistance to foot-rot.

Tissue culture program for selection of heart-rot resistance of A. mangium may require 2 - 3 years of research in the laboratory prior to field assessment.

Figure 8 Tissue culture selection for heart-rot disease resistance of *A. mangium*



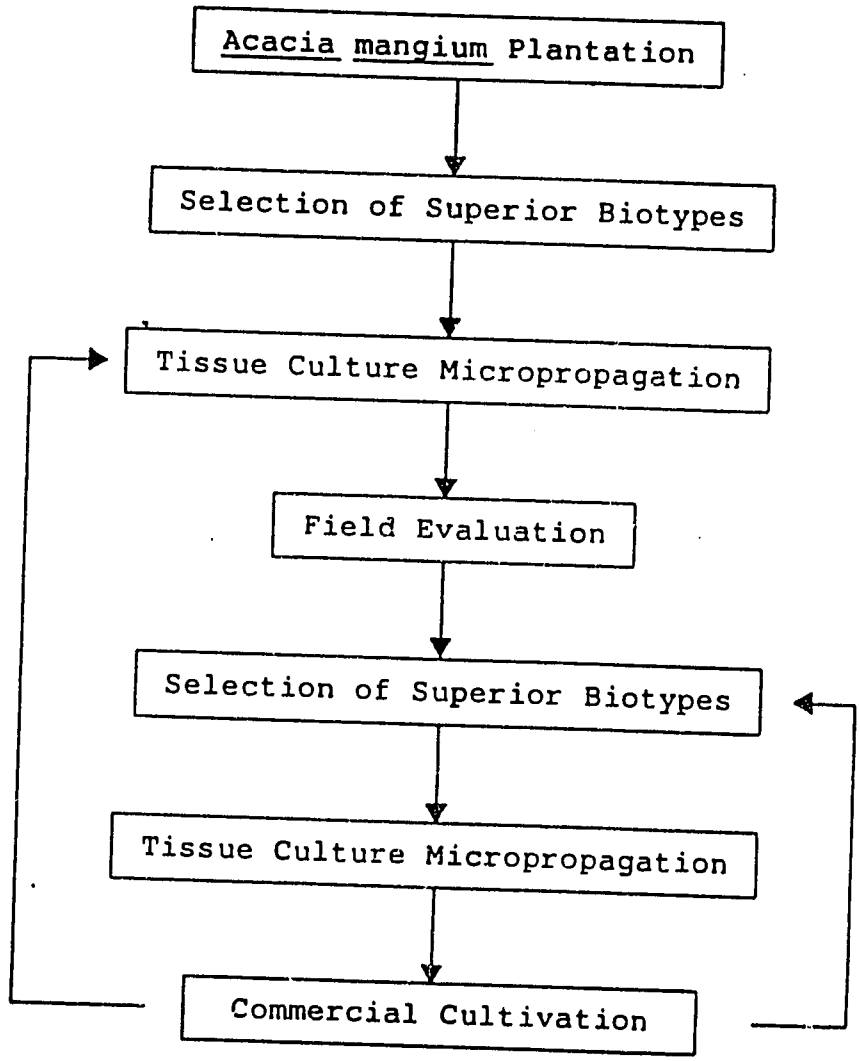
3.2.2 Seed-based breeding program

Breeding programs need to be enhanced to supply the market with high quality seeds (i.e. yielding high-quality trees). Superior trees must be identified either from provenance trials or purchased from Sabah and propagated by marcotting in seed orchards. There are few technical problems and the program could begin to supply the market within 3 - 4 years.

3.2.3 Tissue culture micropropagation

There is currently a gap in the market in that there are no cheap, high quality seeds. A tissue culture program could supply this market competitive to seed prices in the Philippines quoted at S$2,500/kg but not at the price quoted to us in Indonesia (S$600/kg). To use tissue culture, the main technical problem is in the scaling-up procedure. Although tissue culture protocols have already been established at UPM, Malaysia; RFD, Thailand and at BIOTROP, Indonesia, these laboratory protocols have to be refined or modified in order that a large number of plants could be produced economically and efficiently. Another technical problem in the program is that data on field assessment is lacking. Close co-operation with the breeder is required. Details of the suggested program is given in Figure 9. Tissue culture micropropagation may take two years to produce ramets for outplanting.

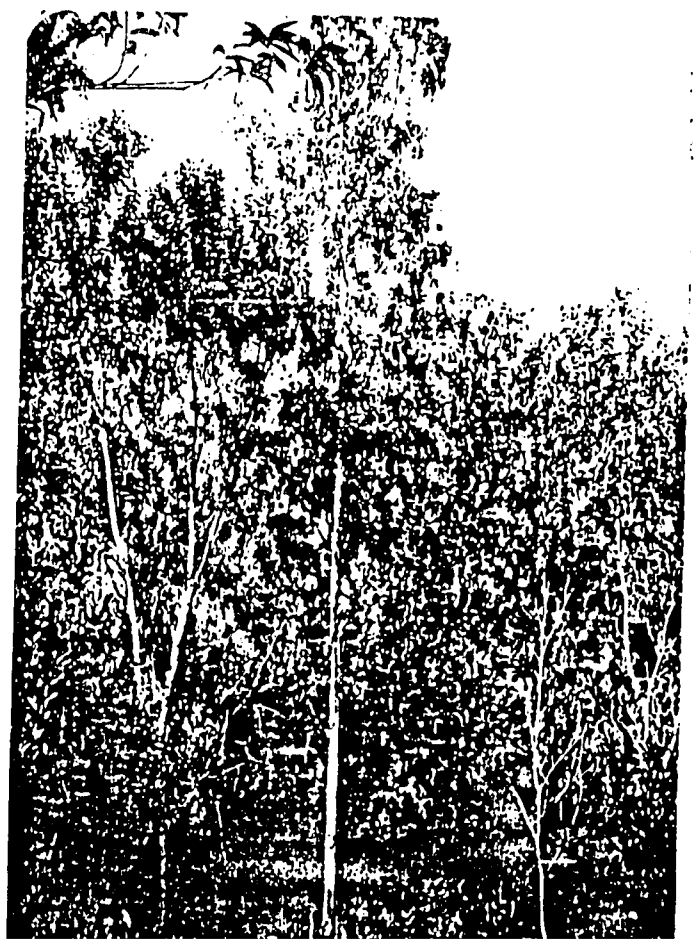
Figure 9: Selection and tissue culture micropropagation of superior biotypes *A. mangium*



3.3 ACACIA AURICULIFORMIS

A. auriculiformis suffers from generally poor stem form in all ASEAN countries except parts of Thailand. In the region it is therefore considered only as fuelwood species. However it has fewer though heavier branches than in A. mangium and there are some trees of good stem form in Thailand (see Picture 1). There is a seed-orchard based on these superior trees but seeds are in short supply. Vegetative propagation by cuttings is difficult so there is prospect for tissue culture of superior biotypes. Although there is little published work, progress in establishing tissue-culture protocols at RFD, Thailand, seems promising. The hybrid between A. mangium and A. auriculiformis shows promise (Sim, 1987), but hybrid seed will be expensive to produce by controlled-pollination and mass-propagation by tissue culture would seem a viable proposition.

Picture 1: Tree of superior stem form growing among more typical A. auriculiformis in a plantation at Sai Thong, Thailand.



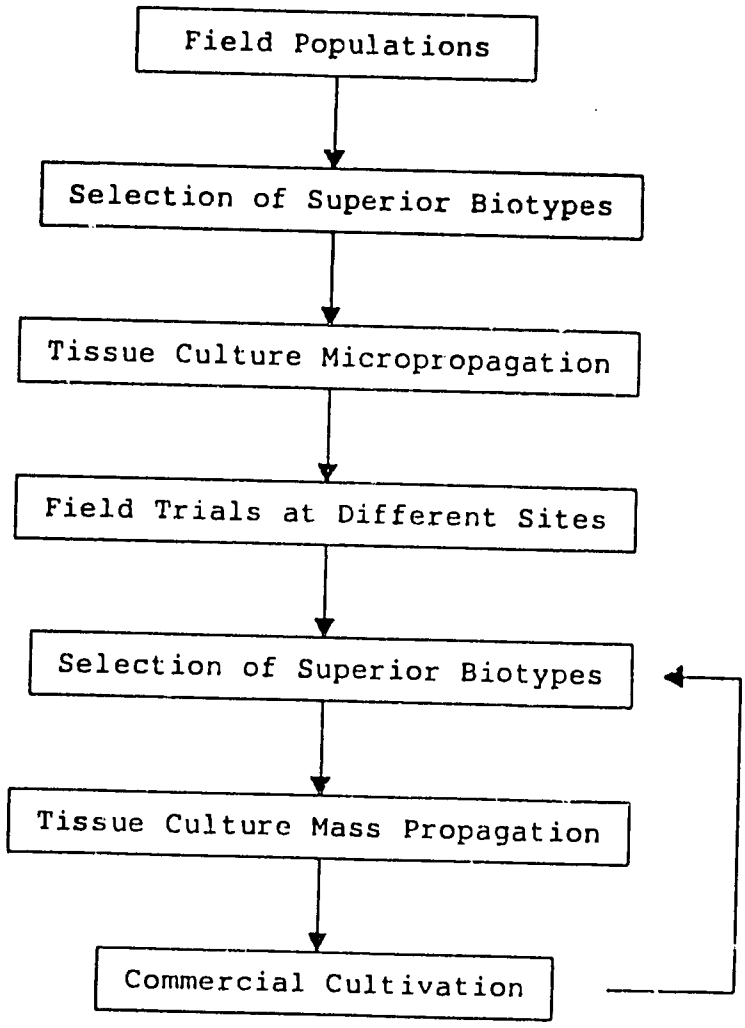
3.3.1 Tissue culture micropropagation of *A. auriculiformis*

Details of the program are similar to 3.2.3 (*A. mangium*). For *A. auriculiformis* tissue culture, final details of protocols are still required and scale-up procedures need developing. The flow chart is as in Figure 10.

3.3.2 Tissue culture micropropagation of hybrid *A. mangium* x *A. auriculiformis*

Hybrid seeds are in very short supply although some are available in Sabah (Sim, 1987) and some will become available at FRIM, Malaysia. There are dangers in hybrid breeding programs because F_2 and succeeding generations will be much more diverse than F_1 or either parent. Also at the present moment, it has not been ascertained whether the hybrid is fertile or sterile. If the hybrid is fertile, it would be preferable to use the best *A. mangium* and *A. auriculiformis* parents available now and produce hybrid seed for testing in the field. Identified superior trees could be micropropagated for distribution and crossed for the next generation. If the hybrid is not fertile, parallel breeding and selection programs should be pursued for both species and the hybrid produced at each generation for testing, selection and micropropagation. There are few technical problems in producing the hybrid and research is already in progress at FRIM and UPM in Malaysia. Tissue culture protocols have yet to be established for the hybrid although they would likely be similar to the parent species. Scale-up procedures would also be required.

Figure 10: Tissue culture micropropagation of *A. auriculiformis* superior biotypes



3.4 EUCALYPTUS CAMALDULENSIS

Selection of appropriate genetic material for propagation whether by seed or by tissue culture is vital for this species. There is wide variation between and within provenances in field trials and superior trees are observable. The genetic basis of this superiority is unknown as there are no progeny tests in the region. Only a proportion of phenotypically superior trees selected now will produce superior clones via tissue culture. There appear to be few technical problems for tissue culture propagation, but vegetative propagation via cuttings from mature trees is very difficult (White, 1986). Tissue culture has two prospects; rejuvenation of mature selected plants for mass propagation by cuttings, and, mass propagation via tissue culture using a bioreactor system.

There are two uses for which micropropagation can be employed.

3.4.1 Tissue culture micropropagation and mass production by bioreactor

Micropropagation of superior biotypes is a possible source of plantlets to fill the gap before large quantities of seeds are available from seed orchards (perhaps in 10 years). Bioreactor/fermentor technology may later be cheaper than seed orchards although the economics are difficult to assess at this stage (Figure 11).

3.4.2 Selection of specific superior traits through tissue culture

E. camaldulensis is well-adapted to a wide

range of sites and this adaptability has been greatly improved for salty sites through tissue culture in Australia (Hartney, 1986). A collaborative program could make use of these clones for salt-affected areas in the region. Further selection for salt tolerance and tolerance to acidity and/or drought could be carried out *in vitro*. Techniques developed in 3.4.1 may well be used to supply large numbers of plantlets cheaply from these selected clones (Figure 12).

Figure 11: *Eucalyptus camaldulensis*: tissue culture micropropagation and mass production by bioreactor

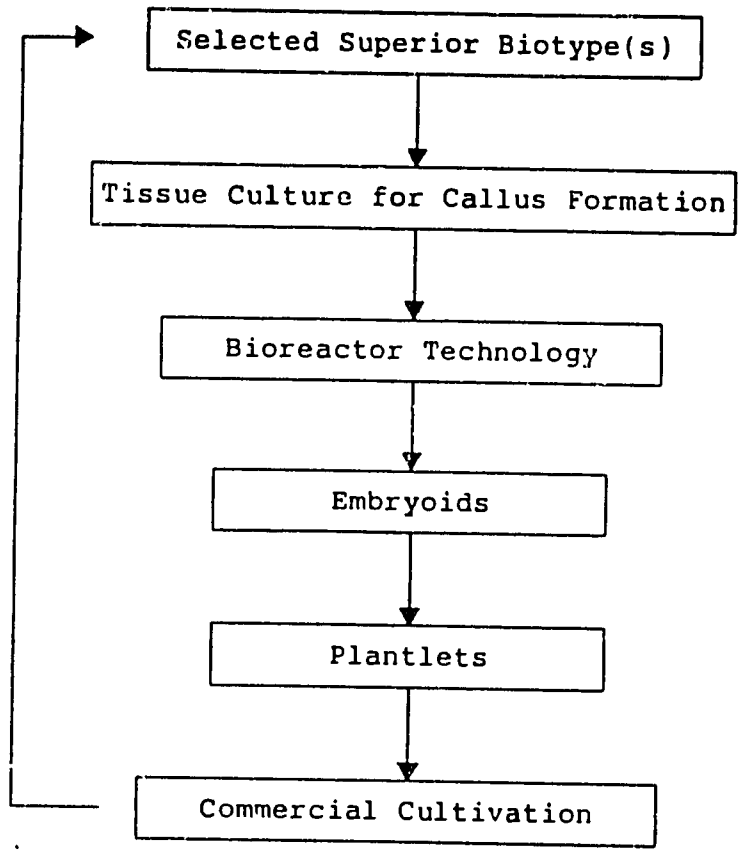
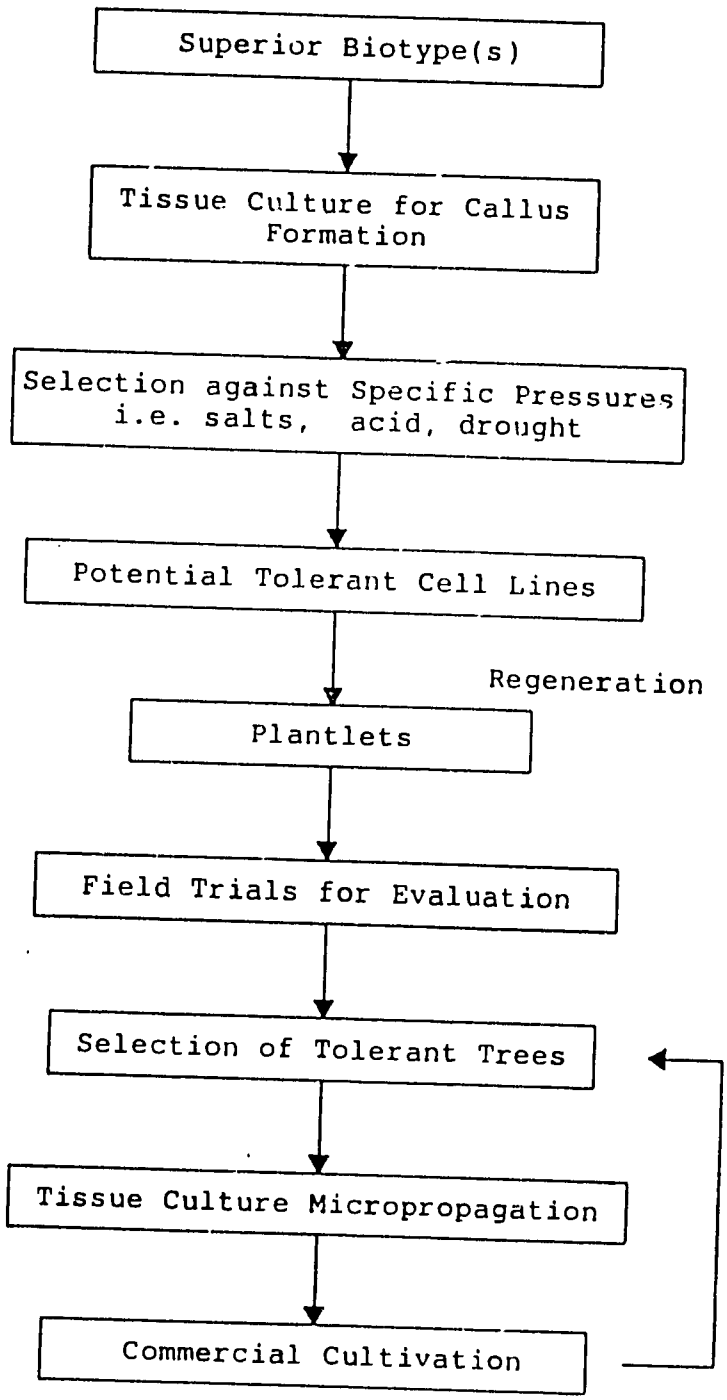


Figure 12: *Eucalyptus camaldulensis*: selection of specific biotype (s)



3.5 DALBERGIA SISSOO

D. sissoo produces a very valuable timber and the seeds are in short supply. When mature, vegetative propagation by cuttings or marcotting is difficult. Tissue culture could provide a good tool for mass propagation of superior mature trees once these can be reliably identified. There seems to be no breeding program in the region to serve as a source of reliably-identified genetically superior trees. According to the literature, there are relatively few problems with tissue culture. There are several laboratories working on the species and some plantlets have been transferred to the field (White, 1986). Scaling-up techniques would need to be established before the process could be carried out commercially. The economics of such a project depend on the costs of the scaling-up process and the price tissue culture plantlets could command in competition with seeds.

3.6 MELIA AZEDARACH, AZADIRACHTA INDICA AND ACACIA NILOTICA

In view of the lack of breeding programs, profusion of seeds available and lack of widespread interest in these species, we do not believe tissue culture should be contemplated for these species.

IV RECOMMENDATION FOR RESEARCH AND DEVELOPMENT PROGRAM

1. LEUCAENA LEUCOCEPHALA

We recommend that:

1.1 Psyllid-resistant plants be urgently identified and that scale-up procedures for tissue culture micropropagation of them be developed.

1.2 An integrated breeding and tissue culture program be initiated immediately (see page 25, sec. 3.1.1).

1.3 As a long-term plan, a genetic engineering selection program be initiated.

2. ACACIA MANGIUM

We recommend that:

2.1 The microorganism, the causal organism for heart-rot, be studied immediately.

2.2 Superior biotypes be identified and that scale-up procedures for large-scale propagation by tissue culture be established.

2.3 An *in vitro* cell selection program for heart-rot resistance be initiated as soon as the microorganism is identified and understood.

2.4 Out planting of ramet be done immediately in order to obtain field assessment of tissue culture plants.

3. ACACIA AURICULIFORMIS

We recommend that:

3.1 Continued identification and selection of superior biotypes and progeny test of superior stem-form trees be carried out.

3.2 Tissue culture protocols and scale-up procedures be established for superior biotypes.

3.3 Hybridization and field testing of hybrid A. mangium x A. auriculiformis to assess potential for tissue culture be carried out.

3.4 Tissue culture protocols (and scale-up procedures) for the hybrid be established after superior hybrids are identified.

4. EUCALYPTUS CAMALDULENSIS

We recommend that:

4.1 Superior biotypes be identified and that scale-up procedures through tissue culture be developed for large-scale multiplication.

4.2 Cell selection program for tolerance to salt, drought and acidic conditions be established.

5. DALBERGIA SISSOO

We recommend that:

5.1 Tissue culture protocols must be developed further and

refined.

5.2 Superior biotypes be identified and that tissue culture scale-up procedures need to be developed.

6. MELIA AZEDARACH; AZADIRACHTA INDICA; ACACIA NILOTICA

No specific tissue culture program recommended at present.

V SUMMARY

1. Literature review on tissue culture of F/FRED priority species (assigned species) suggested that tissue culture protocols have been developed for the following species; Leucaena leucocephala, Acacia mangium, Eucalyptus camaldulensis. Tissue culture protocols of the following species have been partially developed; Acacia auriculiformis, Dalbergia sissoo, Melia azedarach and Azadirachta indica. Acacia nilotica is the only one species where tissue culture protocol has not been found.

2. We found that the following laboratories have been working on tissue culture of one or more species of interest. Laboratories include:

   Plantek International (Pte) Ltd, Singapore
   University Pertanian Malaysis, Malaysia
   Royal Forest Department, Thailand
   BIOTROP, Indonesia
   Forest Research Institute, Philippines
   University of Philippines (Manila), Philippines

3. Though tissue culture programs have been developed in various laboratories, specific goals or objectives are generally not clearly identified.

4. Tissue culture technology for micropropgation has been found technically feasible for application to F/FRED priority species. However, the economics against time must be carefully evaluated for each species. For instance, tissue culture micropropagation may be economically feasible for A. mangium now due to the short supply of quality seeds and seeds are expensive. Once quality seeds are available cheaply, tissue culture may not be economically feasible.

5. We found that tissue culture is a valuable tool for micropropagation of planting materials to complement seed production (not as a substitution) when good quality seed is inadequate and expensive.

6. We found that tissue culture is a viable tool to encounter existing problems i.e. selection for psyllid resistance in Leucaena, improve yield of Acacias and Eucalyptus, selection for heart-rot resistance in Acacia mangium, etc.

7. Using tissue culture in the breeding program of F/FRED priority species will indeed speed up the program, shorten the time and provide adequate experimental materials for field trial (progeny trial) and selection.

8. Though tissue culture has been reported successful in some of F/FRED priority species, field assessments of tissue culture plantlets have not been established. The need to obtain field information is urgent.

9. We recommend F/FRED to include tissue culture micropropagation and improvement in the program as soon as possible. Though F/FRED program is focused to assist small farmers, tissue culture micropropagation fits well into the program because when taken as a whole, the quantity of planting material required is large. Tissue culture once developed can meet demands for both quantity and quality.

10. We suggest for F/FRED to set up a tissue culture network for forest species in this region by funding, monitoring and coordinating a research and development program in various laboratories in the region.

APPENDIX 1

LITERATURE REVIEW: TISSUE CULTURE

Tissue culture technological applications to horticultural and agronomical crops have been extensively reviewed. Successful applications of this technology to agriculture have been widely observed but to a lesser extent for forest trees. Tissue culture of forest trees has been actively explored using embryos, hypocotyl of the seedlings, shoots, meristem, etc. as the explant source. Table 2 shows the gymnosperm species which have been studied in tissue culture. Table 3 shows some woody dicotyledonary forest tree species from which plantlets have been regenerated from tissue or organ culture.

1. Leucaena leucocephala (Lam.) de Wit.

Tissue culture of L. leucocephala (Lam) de Wit has been reported since 1980 by Peasley and Collins who suggested using tissue culture system for Leucaena. Work was mainly done using seedling tissues such as cotyledonary tissue and hypocotyl (Venketeswaran and Romano, 1982; Nagami and Venketeswaran, 1983; Nataraja and Sudhadevi, 1984; Venketeswaran et. al., 1984)

Success of tissue culture from mature tree tissue has been recently reported (Goyal et. al, 1985; Datta and Datta, 1984). Tissues taken from 2-3 m tall greenhouse grown trees (Goyal et. al, 1985) and from 'elite' trees have been successfully cultured and multiplied rapidly on modified MS medium supplemented with growth regulators, NAA and BA. Root induction was readily obtained by culturing shoots on 0.05 mg/l kinetin and 1 mg/l IBA.

L. diversifolia has also been tissue cultured. Regeneration of callus has been successful. Protoplasts of both species

have been isolated with limited success (Venketeswaran et. al., 1984).

2. Acacias

Reports on tissue culture of Acacia mangium and Acacia auriculiformis have not been found so far. Tissue culture of other Acacias such as A. senegal (Kathju and Tewari, 1973; Hustache, et. al. 1986) A. koa (Skolmen and Mapes, 1976) and A. georginae-D (Preuss, et. al., 1970) has been reported. Most of the work used seedling explants.

However, during our survey, successful tissue culture of A. mangium was observed at UPM, Malaysia; RFD, Thailand and BIOTROP, Indonesia. Tissue culture of A. auriculiformis has been experimented at RFD, Thailand. Results are very encouraging.

3. Eucalyptus camaldulensis

Tissue culture of Eucalyptus spp. has been studied since the 1960's using tissues from both in vivo and in vitro grown seedlings. Callus was induced and maintained for a long period of time without appa;rent changes in ploidy level (Sussex, 1965; Piton, 1969). Regeneration of plantlets from callus via embryogenesis was successful (Ouyang, et. al, 1981).

Tissue culture of Eucalyptus spp. from mature trees has been difficult but successful in some species. For instance, Cresswell and Nitsch (1975) reported the successful culture of tissue taken from mature E. grandis. Similarly, Gupta, et. al. (1983) reported the successful culture of tissue from a mature E. torelliana and E.

camaldulensis. High multiplication rate and ready rooting have been reported by using a combination of rejuvenated adult tissues by coppicing and rooting of coppice shoot cuttings. These cuttings which are then pinched to induce basal branching, provide material for nodal cultures (Durand - Cresswell et. al. 1985).

Several problems encountered on culturing mature tissues included high contamination due to endogenous bacterial contaminants (De Fossard et. al., 1977), rooting inhibitors (Piton et. al., 1970), bud inhibitors (Blake and Carrodus, 1970) and maturity of the tissue (adult-phase stage) (Cresswell and Nitsch, 1975).

Though many problems have created difficulties for Eucalyptus tissue culture, success has been accomplished in E. camaldulensis for mass propagation. Mature selected trees could be mulitplied rapidly, rooted and transplanted to the soil successfuly (Duran - Cresswell et. al., 1985).

4. Dalbergia sissoo

Success of Dalbergia tissue culture has been reported in 1982. Datta and Datta (1983) and Datta, et. al., 1982 reported that Dalbergia sissoo tissue from seedlings and from mature trees (30 years old) could be cultured under aseptic condition. Multiple shoots could be produced. They suggested that this technique could be used for rapid clonal multiplication. At the same time, Nataraja and Sudhadevi (1984) reported successful culture of Dalbergia latifolia seedling explants. In 1984, success of Dalbergia sissoo tissue culture has also been reported (Datta and Pramanik, 1984; Dawra et. al., 1984; Mukhopadhyay and Ram, 1984). Anand and Bin (1984) has reported the successful culture of Dalbergia lauceolaria.

BIOTROP, Indonesia, is now working on tissue culture of Dalbergia latifolia, so far with limited success.

5. Azadirachta indica (neem)

Most of the work towards tissue culture of Azadirachta indica has been for secondary metabolite(s) i.e. pesticidal chemicals rather than micropropagation. Work has been undertaken in the 1980's. Successful regeneration of plantlets from the somatic tissue of neem has been reported (Jaiswal and Narayan, 1983). Using tissue culture for the biosynthesis of Nimbin or for the study of Nimbin biosynthesis have been the main focus of tissue culture research (Sanyal and Data, 1981; Sanyal and Datta, 1986; Sanyal, et. al., 1981; Schulz 1983).

6. Melia azedarach

Tissue culture of Melia azedarach has been preliminarily worked out in 1983 at Kasetsart University (Vilash, 1983). Limited success has been reported. Project was not continued.

Table 2 : *In vitro* cultures of gymnosperms

Species	Type of explant	Type of development
CYCADALES		
Cycas circinalis	Megagametophyte callus	embryogenesis
Zamia floridana	Megagametophyte	Shoot formation
Zamia integrifolia	Proembryo callus Megagametophyte Embryo	Embryogenesis Embryogenesis Embryogenesis
CONIFERALES		
Pinaceae		
Abies balsamea	Shoots	Axillary bud formation, embryogenesis
	Buds	Shoot formation
Laris x *eurolepis*	Stem segment	Axillary bud formation
Picea abies	Cell suspension Embryo callus Embryo Hypocotyl Needle Bud Shoot	Embryogenesis Shoot formation Shoot formation Shoot formation Shoot formation Shoot formation Root formation Plant in soil
Picea glauca	Hypocotyl Needle Bud Shoot	Shoot formation Embryogenesis Shoot formation Root formation, plant in soil
Picea mariana	Bud	Shoot formation
Picea sitchensis	Embryo callus Embryo Cotyledon Shoot	Shoot formation Shoot formation Shoot formation Root formation
Pinus banksiana	Hypocotyl	Shoot formation
Pinus contorta	Cotyledon Embryo Shoot	Shoot formation Shoot formation Root formation

Species	Type of explant	Type of development
Pinus gerardiana	Subcultured tissues	Shoot and root formation
Pinus palustris	Embryo	Shoot and root
	Shoot	Root formation, plant in soil
Pinus pinaster	Apex with cotyledons	Axillary bud formation
	Brachyblast with needles	Apical bud formation
	Cotyledon	Shoot formation
	Embryo	Shoot formation
	Bud	Shoot formation
	Shoot	Root formation, plant in soil
Pinus radiata	Embryo callus and juvenile needles	Shoot formation
	Cotyledon callus	Shoot formation
	Embryo	Shoot formation
	Cotyledon	Shoot formation
	Needles	Shoot formation
	Shoot	Root formation, plant in soil
Pinus resinosa	Embryo	Embryogenesis
Pinus sylvestris	Cotyledon	Shoot formation
	Brachyblast with needles	Shoot formation
	Shoot	Root formation, plant in soil
Pinus strobus	Embryo callus	No organogenesis
	Embryo	Shoot formation
	Shoot	Root formation
Pinus taeda	Cell suspension	Embryogenesis
Pinus wallichiana	Embryo	Shoot formation
	Embryo callus	Shoot formation
	Bud	Shoot formation

Species	Type of explant	Type of development
Pseudotsuga menziesii	Subcultured tissues	Shoot formation
	Embryo callus	Shoot formation
	Cotyledon callus	Shoot formation
	Cell suspenson	Emryogenesis
	Bud	Axillary bud formation
	Embryo	Shoot formation
	Cotyledon	Shoot formation
	Needles	Shoot formation
	Bud	Shoot formation
	Shoot	Root formation
		Plant in soil
Pseudotsuga taxifolia	Bud	Shoot formation
Tsuga heterophylla	Cotyledon	Shoot formation
	Shoot	Root formation, plant in soil
Taxodiaceae		
Cryptomeria japonica	Hypocotyl	Shoot formation
Sequoia gigantea	Cotyledon	Shoot formation
Sequoia sempervirens	Subcultured tissue	Shoot formation
	Stem segment	Axillary bud formation
	Bud	Shoot formation
	Shoot	Root formation, plant in soil
Cupressaceae		
Biota orientalis	Hypocotyl callus	Shoot and root formation
	Hypocotyl, embryo	Shoot formation
	Embryo	Embryogenesis
Cupressus arizonica	Cotyledon	Shoot formation
Thuja plicata	Cotyledon	Shoot formation
	Leaves	Shoot formation
	Shoot	Root formation, plant in soil

Source: David, A. 1985. *In vitro* propagation of Gymnosperms. In: Tissue Culture in Forestry. J.M. Bonga and D.J. Drurzan, eds. Martinus Nijhoff/Dr W.Hunk Publishers. pp. 105-108.

Table 3: **Some woody dicotyledonous forest tree species from which plantlets have been regenerated from tissue or organ culture.**

SPECIES	TREES AGE[a]	EXPLANT SOURCE
Acacia koa	J	Shoot tip
Acer rubrum	J	Shoot tip, axillary buds
	-	Shoot tip, node sections
Actinidia chinensis	-	Stem, root segments
A. chinensis var. chinensis var. hispida	M	Stem segments
Albizzia lebbeck	J	Hypocotyl
Alnus glutinosa	J	Axillary buds
	J	Shoot tip
A. rubra	J	Shoot tip, buds, hypocotyls
Amelanchier laevis	-	Shoot tip
Atriplex canescens	J	Shoot tip
Betula papyrifera	J	Buds
B. pendula	J	Stem
	J	Stem
	-	Stem
	M	Catkins
B. platyphylla 'Schezuanica'	J	Buds
	J	Internodes from shoot tip cultures
	J,M	Shoot tips
Brachycome sp.	-	Leaf, stem, cotyledons
Broussonetia kazinoki	J	Hypocotyl
Camellia	J	Shoot tip in vitro grafting
Carya illinoensis	J	Axillary buds
Castanea dentata	J	Embryo culture
C. sativa	J	Embryonic axes
	J	Lateral buds
Catalpa bignonioides	J	Shoot tip
Cinchona ledgerana	J	Shoot tip
Corylus avellana	J	Embryos
	J	Embryonic axes
Diospyros kaki	J	Hypocotyl
Eucalyptus alba	J	Hypocotyl
E. bancrofti	J	Shoot tip
E. camaldulensis	J	Shoots
	-	Node

SPECIES	TREES AGE[a]	EXPLANT SOURCE
E. citriodora	J	Hypocotyl, cotyledons
	M	Terminal buds
	-	Lignotubers
E. curtisii	J	Shoots
E. dalrympleana	J	Apex, axillary buds
	J	Nodes
E. deglupta	J	Nodes, shoot tip
E. ficifolia	J,M	Nodes
E. globulus	J	Shoots
E. grandis	J	Nodes
	J	Shoots
	J	Nodes, shoot tip
	-	Nodes
E. gunnii	J	Apex, axillary buds
E. nitens	J	Shoots
E. obtusifolia	J	Shoots
E. orcades	J	Shoots
E. pauciflora	J	Shoots
	J	Apex, axillary buds
E. regnans	J	Shoots
	J	Nodes
E. robusta	J	Shoot tip
	-	Nodes
Fagus silvatica	-	Axillary buds
Ficus benjamina	-	Shoot tip
E. decora 'Elastica'	-	Shoot tip
F. lyrata	-	Leaves
F. pandurata	-	Shoot tip
Gleditsia triocanthos var. inermis	J	Shoot tip
G. triocanthos	-	Shoots
	J	Cotyledons
Hamamelia virginiana	J	Embryos
H. vernalis	J	Embryos
Hedera helix	J,M	Callus from stems
Ilex aquifolium	J	Cotyledon
I. cornuta	J	Cotyledon
I. opaca	J	Cotyledon
Kalmia latifolia	J	Shoot tip
Liquidambar styraciflua	J	Shoot tip, hypocotyls
Liriodendron tulipifera	M	Terminal buds
Moghania macrophylla	J	Seed
Morus sp.	J	Hypocotyl

SPECIES	TREES AGE[a]	EXPLANT SOURCE
Paulownia taiwaniana	-	Ovules
	J	Stem
P. tomentosa	J	Hypocotyl
	J	Shoot tip, axillary buds, petiole, leaf base
	M	Unfertilized ovules
Populus alba	-	Axillary buds
	J	Twig
	-	Axillary buds, apical buds
P. alba x glandulosa	-	Axillary buds, apical buds
P. alba x tremula	-	Axillary buds, apical buds
Populus x canadensis	J	Stem tip
P. canescens	J	Cambium, shoot tip
	-	Axillary buds, apical buds
P. euramericana	-	Shoot tip, callus
P. euramericana 'Robusta'	J	Cambium, shoot tip
	-	Axillary buds
	J	Twig
Populus 'Flevo' (P. deltoides x P. nigra)	-	Axillary buds
P. nigra 'Italica'	M	Twigs
P. nigra 'Typica'	J	Cambium, shoot tip
	-	Axillary buds
P. nigra	J	Twig
P. tremula	J	
	-	Axillary buds
	J	Twig
	J	Cambium, shoot tip
	-	Axillary buds, apical buds
P. tremuloides	-	Axillary buds
	-	Cambium
	-	Root sprouts
	J	Stem tip of root sucker
P. tristis x P. balsamifera 'Tristis #1'	-	Shoot apex
P. yunnanensis	-	Axillary buds
Prunus amygdalus	J	Stem
	J	Epicotyl
P. amygdalus and hybrids	-	Shoot tip, dormant shoot buds
	J	Shoot tip
P. avium	M	Buds
Quercus robur	-	Axillary bud

SPECIES	TREES AGE[a]	EXPLANT SOURCE
Rhododendron canescens	-	Shoot tip
R. catawbiense 'Nora Zembia'	M	Florets
R. catawbiense 'Roseum Elegans'	M	Florets
R. prunifolium	-	Shoot tip
Rhododendron spp.	-	Shoot tip, axillary buds
Robinia pseudoacacia	-	Roots
Salix aquatica	-	Lateral buds
S. babylonica	J	Axillary buds
S. interior	-	Lateral buds
S. purpurea	-	Lateral buds
S. viminalis	-	Nodes
Santalum acuminatum	J	Nodes & internodes apex, cotyledons, hypocotyl, roots
S. album	J	Endosperm
	-	Shoots
	J	Hyocotyls
	J	Embryo
	M	Shoot segments, shoot tip
S. lanceolatum	J	Nodes & internodes
Sapium sebiferum	J	Hypocotyl
Simmondsia chinensis	-	Shoot tip
Tectona grandis	M,J	Shoot tip, stem, cotyledons, axillary buds
Tupidanthus calyptratus	-	Shoot tip
Ulmus americana	J	Hypocotyls
U. campestris	J	Twigs
	-	Axillary buds
U. effusa	-	Axillary buds
U. scabra	-	Axillary buds
Vaccinium angustifolium	J	Hypocotyls
	M	Buds
V. corymbosum	-	Shoot tip

[a]M = mature ; J = juvenile

Source: Sommer, H.E and H.Y. Wetzstein. 1985. Hardwoods. In: Handbook of Plant Cell Culture Vol.3. Crop Species. P.V. Ammirato, D.A. Evans. W.R. Sharp and Y. Yamada. Macmillan Publishing Co. pp. 511-540.

APPENDIX 2

REFERENCES

Anand, D. and S.S. Bir, 1984. Organogenetic differentiation in tissue cultures of Dalbergia lanceolaria. Curr. Sci. (Bangalore). 53(24):1305-1307.

Benge, M.D.,1982. The miracle tree : reality or myth. "Leucaena research in the Asian-Pacific region" Proc IDRC Workshop, Singapore, 95-8,IDRC: Ottawa.

Biondi, S. and T.A. Thorpe., 1981. Clonal propagation of forest tree species. In : Proc. COSTED Symp. on Tissue Culture of economically important plants. pp. 197-204.

Blake, T.J. and B.B. Carrodus, 1979. Studies on the lignotubers of Eucalyptus obliqua l'Herit. II Endogenous inhibitor levels correlated with apical dominance. New Phytol 69:1073-1079.

Bonga, J.M., 1981 Vegetative propagation of mature trees by tissue culture. In : Proc. COSTED. Symp. on Tissue Culture of economically important plants. A.N. Rao, ed. pp. 191-196

BOSTID, 1980. "Firewood Crops - Shrub and Tree Species for Energy Production " report of an ad hoc panel of the Advisory Committee on Technological Innovation, Board on Science and Technology for International Development (BOSTID). National Academy of Sciences : Washington DC.

BOSTID, 1983. "Mangium and other fast-growing Acacias for the Humid Tropics" report of ad hoc panel of Advisory Committee on Technology Innovation, Board on Science and Technology for International Development (BOSTID). National Academy of Sciences : Washington DC.

BOSTID ,1983b. "Firewood Crops- Shrub and Tree Species for Energy Production " Vol 2. report of an ad hoc panel of the Advisory Committee on Technological Innovation, Board on Science and Technology for International Development (BOSTID). National Academy of Sciences : Washington DC.

BOSTID, 1984. "Leucaena : Promising Forage and Tree Crop for the Tropics" report of ad hoc panel of Advisory Committee on Technology Innovation, Board on Science and Technology for International Development (BOSTID). National Academy of Sciences : Washington DC.

Brewbaker, J.L.,1982. Systematics, self-imcompatibility, breeding systems, and genetic improvement of Leucaena species. "Leucaena research in the Asia-Pacific region". Proc IDRC Workshop, Singapore, 17-22,IDRC: Ottawa.

Cresswell, R. and C. Nitsch, 1975. Organ culture of Eucalyptus grandis L. Planta (Berl.) 125: 87-90.

Committee, 1985. Tropical Forest Action Plan. FAO. Rome. pp. 159

Datta, S.K. and K. Datta, 1983. Auxin induced regeneration of forest tree Dalbergia sissoo Roxb. through tissue culture. Current Science. 52(9):434-436.

Datta, S.K and K. Datta, 1984. Clonal multiplication of 'Elite' trees -Leucaena leucocepala through tissue culture. Leucaena Research Reports. 5: 22-23.

Datta, S.K., K. Datta, and T. Pramanik,1982. In vitro clonal multiplication of mature trees of Dalbergia sissoo Roxb. (Tissue culture, micropropagation). Plant cell, Tissue and Organ Culture. 2(1):15-20.

Datta, S.K. and T.K. Pramanik, 1984 — In vitro response of exogenous growth regulators on endogenous IAA induced xylogenesis and regeneration potential of timber tree - Dalbergia sissoo Robx. Proceedings, Pacific Regional Wood Anatomy Conference, October 1-7, 1984, Tsubuka, Ibaraki, Japan (Edited by Sudo, S.).

David, A., 1985. — In vitro propagation of Gymnosperms. In: Tissue Culture in Forestry. J.M. Bonga and D.J. Drerzon , eds. Martinus Nijhoff/Dr. W.Junk. Publishers. pp. 72-108.

Dawra, S., D.R. Sharma and J.B. Chowdhury,1984. — Clonal propagation of Dalbergia sissoo through tissue culture. Curr. Sci. (Bangalore). 53(13):807-809.

De Fossard, R.A., P.K., Barker and R.A. Bousre, 1977. — The organ culture of nodes of four species of Eucalyptus. Acta Hortic. 78: 157-165.

Durand-Cresswell, R. and C. Nitsch, 1977. — Factors influencing the regeneration of Eucalyptus grandis by organ culture. Acta Hortic. 78: 149-155.

Durand-Cresswell, R., M. Boulay and A. Franclet, 1985. — Vegetative propagation of Eucalyptus. In : Tissue Culture in Forestry. J.M. Bonga and D.J. Drerzon, eds. Martinus Nighoff / Dr. W. Junk. Publishers. pp. 150-181.

FAO,1987. Report of the expert consultation on use of tissue culture in plant quarantine for exchange of germplasm and planting materials. FAO Regional Office for Asia and the Pacific, Bangkok.

Goyal, Y., R.L. Bingham, and P. Felker, 1985. Propagation of the tropical tree, Leucaena leucocephala K67, by in vitro bud culture. Plant Cell, Tissue and Organ Culture. 4(1):3-10.

Gupta, P.K., U.J. Mehta and A.F.Mascarenhas, 1983. Tissue culture method for rapid clonal propagation of mature trees of Eucalyptus torelliana and Eucalyptus camaldulensis Plant Cell Reports . 2 : 296-299

Gupta, P.K. and D.J. Drurzan,1987. Biotechnology of somatic polyembryogenesis and plantlet regeneration in Loblolly pine. Bio/Technology. 5 : 147-151.

Hartney, V.J.,1982. Tissue Culture of Eucalyptus. Int. Pl. Prop. Soc. combined Proc. 32: 98-109.

Hartney,V., 1986. Vegetative propagation of Eucalypts. Aust. For Res. 10, 191-211.

Hartney, V.J and E.D. Kabay, 1984. From tissue culture to forest trees. In: Pl. Prop. Soc. Combine Proc. 34: 93-99.

Hustache, G., F. Barnoud and J.P. Joseleau,1986. Callus formation and induction of a cell suspension culture from Acacia senegal. Plant Cell Rep., 5:365-367.

Jaiswal, V.S. and P. Narayan, 1983. Regeneration of plantlets from the somatic tissue of some Indian trees. Proceedings of National Symposium on Advancing Frontiers of Plant Sciences, Jodhpur, Nov. 26-30, 1983. Edited by H.C. Arya et. al. p.138-139.

Kathju, S. and M.N. Tawari,1973 Development of the root from the cotyledonary callus of Acacia senegal. Labdev. J. Sci. Technol. Part B Life Sci. 11(3-4):84-85.

Lee, S.S., 1986. Discoloration of the heartwood of Acacia mangium - A preliminary report. Proc 2nd Int. Conf. Pl. Prot. in the tropics, P-02,331-2.

Mascaranhas, A.F., P.K. Gupta, V.M. Kulkarni, U. Mehta, R.S. Lyer, S.S. Khuspe and V. Jagannathan,1981. In : Proc. COSTED Symp. on Tissue Culture of Economically Important Plants. A.N. Rao, ed. pp. 175-184.

Matheson, A.C.,and D. Lidgren, 1985. Gains for the clonal and the clonal seed-orchard options compared for tree breeding programs. Theor. Appl. Genet. 71, 242-9.

McKeand, S.,1981. Lobolly pine tissue cultures : present and future uses in southern forestry. Tech Rep No. 64, School of For. Res. North Carolina State University : Raleigh.

Mukhopadhyay, A. and H.Y.M. Ram, 1984. Regeneration of plantlets from excised roots of Dalbergia sissoo. Indian J. Exp. Biol. 19(12):113-115.

Nagami, R. and S. Venketeswaran, 1983. Morphological responses of cultured hypocotyl and cotyledonary segments of Leucaena. In Vitro 19(3):265.

Nataraja, K., and A.M. Sudhadevi,1984. In vitro induction of plants from seedling explants of subabul (Leucaena leucocephala lamk.) Indian. J. of. Pl. Phys. 27(3): 255-258

Ouyang Q. H.Z. Peng and Q.Q. Li,1981. Studies on the development of embryoids from Eucalyptus callus. Scientia Silval Sinicae. 17 : 1-7

Peasley, E.L and G.B. Collins, 1980. Development of an in vitro culture system for Leucaena. Leucaena Newsletter. 1 : 54

Piton, F., 1969. Quelqnes observations cytologiques sur des tissue d'Eucalyptus camaldulensis cultives in vitro. Rev. Gen. Bot. 76 : 287-307.

Preuss, P., R. Birkhahn, and E.D. Bergmann,1970. The effect of sodium fluoride on the growth and metabolism of tissue cultures of Acacia georginae - D and tomato - D. Israel J. Bot. 19(4):609-619.

Rajadurai,D.D.,1987. In vitro studies on some woody legumes (In press).

Schulz, F.A., 1983. Tissue culture of Azadirachta indica. Proceedings of the International Neem Conference, No. 2. Edited by Schmutterer, H. and Ascher, K.R.S. Natural pesticides from the neem (Azadirachta indica A. Juss) and other tropical plants; Ranischholzhauser, West Germany, May 25-28, 1983. 587p. Deytsche Gesellschaft Fuer Technische Zusammenarbeit GMBH, Eschborn, West Germany. Illus. ISBN 3-88085-156-5. 1984.

Sedgley,M., 1986. Reproductive biology of Acacias. "Australian Acacias in Developing Countries" Proc Int Workshop, Gympie, Queensland, Australia ed J.W Turnbull. ACIAR : Canbena

Sanyal,M., A. Das, M. Danerjee, and P.C. Datta,1981. In Vitro hormone induced chemical and histological differentiation in stem callus of neem Azadirachta indica. A. Iuss (Triterpenes). Indian Journal of Expermental Biology. 19(11):1067-1068.

Sanyal, M. and P.C. Datta, 1981. Nimbin biosynthesis and the age of cultured callus from neem bark (Azadirachta indica). Indian Drugs. 19(2):61-63.

Sanyal, M. and P.C. Datta, 1986. Nutritional environment and redifferentiation from cultured tissues of Azadirachta. Acta Horticultural. No. 188:99-103.

Skolmen, R.G. and M.O. Mapes, 1976. Acacia koa plantlets from somatic callus tissue. J. Hered. 67(2):114-115.

Sim, B.L., 1984. The genetic base of Acacia mangium Willd, in Sabah. Proc IUFRO Symp. " Provenance and genetic improvement stategies in tropical forest trees " Mutare, Zimbabwe, April 1984 (eds R.D Barnes and G.L Gibson) 597-603, O.F.I : Oxford.

Sim, B.L., 1987. Present research status on hybridisation and vegetative propagation of A. mangium and A. auriculiformis. Unpublished draft.

Sommer, H.E and H.Y. Wetzstein,1985. Hardwoods. In : Handbook of Plant Cell Culture. Vol.3. Crop Species. P.V. Ammirato, D.A. Evans, W.R. Sharp and Y. Yamada. Macmillan Publishing Co. pp. 511-540.

Sussex, I.M., 1965. The origin and morphogeneses of Eucalyptus cell population. In : Proceedings of an International Conference on Plant Tissue Culture. P.R White and A.R Grove, eds. McCutcheon Publ. Co., Berkley, pp. 383-391.

Turnbull,K.W.,1986. "Multipurpose Australian Trees and Shrubs - lesser known species for fuelwood and agroforestry." ACIAR : Canbena.

Venketeswaran, S. and E.J.Romano, 1982. Tissue culture of forest trees for biomass energy production through in vitro propagation (Sapium sebiferum, Leucaena leucocephala, Capaifera multijugar). Proc. 5th Int. Cong. Pl. Tissue and Cell Cult., Tokyo and Lake Yamanake, Japan. pp. 725-726.

Venketeswaran, S., R. Nagmari and V. Gandhi, 1984. Tissue culture propagation of selected tree genera for biomass bioenergy 85.Proc. Conf., Goteborg, Sweden. Vol.II. Biomass Resources. E.H. Ellegard, ed.

Vilash, V. 1983. Vegetative propagation of Melia azedarach Linn. for the possibility of mass production. Kasetsart Univ., Bangkok (Thailand). Thesis (M.S. in Agriculture). 1983.

White, K.J., 1986. Practical tree breeding strategies and practices and programmes in the Bhabarterai of Central Nepal. Manual No. 5. pp. 47.

APPENDIX 3

ITINERARY & USEFUL ADDRESSES

May 4

1. Forest Research Institute of Malaysia (FRIM)
   P.O. Box 201, Kepong, Selangor
   52109, Malaysia

   Dr Francis S.P Ng
   Mr Zakariah Ibrahim (Tree Improvement Officer)

2. Sime Darby Plantations
   Ebor Research
   P.O. Box 147 Jalan Meru
   41050 Klang, Malaysia

   Mr Brian Wood (Research Director)
   Mr Julien Kanagaratnan (Project Manager- tissue culture)
   Ms Wooi Kheng Choo (Biotechnologist- tissue culture)

May 5

3. Rubber Research Institute Malaysia (RRIM)
   260 Jalan Ampang
   50450 Kuala Lumpur, Malaysia

   Dr E Pushparajah (Assistant Director- Dept. Biology)

4. University Pertanian Malaysia (UPM)
   43400, Serdang, Selangor
   Malaysia

   Dr Kamis Awang (Dean, Faculty of Forestry)
   Dr Zaliha Christine Alang (Tissue Culture Lab.,Dept. Food Science & Technology)

May 6

5. Deutsche Gesellschaftfur
   Technische Zusammenarbeit (GTZ)
   Forest Research Institute of Malaysia
   P.O. Box 201, Kepong, Selangor
   52109, Malaysia

   Pro. Dr Jozsef Racz
   Dr Yap Son Kheong

May 7 6. FAO Regional Office for Asia and the Pacific
Phra Atit Road, Bangkok 10200,
Thailand

Dr R.B. Singh (Regional Plant Production & Protection Officer)
Dr Y.S. Rao (Regional Forestry Officer)

7. Division of Silviculture
Royal Forest Department
Bangkhean, Bangkok 10900,
Thailand

Dr Boonchoob Boontawee (Chief)
Mr Khongsak Pinyopusarerk (Research Coordinator)

8. Department of Horticulture
Kasetsart University
Bangkok 10900,
Thailand

Ms Pranom Prutpongse

9. Faculty of Forestry
Kasetsart University
Bangkok 10900,
Thailand

Dr Suree Bhumibhamon (Associate Professor)

May 8 Ratchaburi experimental area of Royal Forest Department

May 11 10. Department of Agronomy
Bogor Agricultural University (IPB)
Bogor, Indonesia

Dr Levy Gunawan

11. Forest Research and Development Center
Ministry of Forestry
P.O. Box 66, Bogor,
Indonesia

Dr Komar Soemarna (Director)

12. South East Asia Regional Center for Tropical Biology (BIOTROP)
Jalan Raya Tajur KM. 6
P.O. Box 17, Bogor,
Indonesia

Dr Tri Binarko Suselo

May 12 13. Faculty of Forestry
Bogor Agricultural University (IPB)
P.O.Box 69, Bogor
Indonesia

Dr Ir. M. Yahya Fakuara Ts
Dr Yadi Setiadi

May 14 14. University of the Philippines
Manila, Philippines

Dr Saturnina Halos

15. Bureau of Forest Development
Diliman, Quezon City,
Philippines

Dr Isidro D. Esteban (Assistant Director General)

16. Manila Seedling Bank Foundation Inc.
Quezon Ave. Ext. cor.E. delos Santos
Quezon City, Philippines

Mr Lucito M. Bertol (Vice President)
Mr Banjamin D. Malto (Head, Planning & Evaluation Dept.)

17. Philippine Wood Products Association
3rd Floor, LIA Building
118 Perca St, Legaspi Village
Makati, Metro Manila,
Philippine

Mr Eulogio T. Taguda (Executive Secretary)

May 15-16 18. National Institute of Biotechnology and Faculty of Forestry and Forest Research Institute (FORI) University of the Philippines at Los Banos College, Laguna, Philippines

Dr Mercedes UmaliGarcia
Dr Cesar C. Nuevo
Dr Eduardo B. Principe (Chief, Nat. For. Tree Seed Res. & Cert. Center)
Mr William R. Palaypayon (Science Research Specialist, Nat. For. Tree Seed Res. Cert. Center)