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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

61771

DATE: 1/4/89

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 8196
PR#1

Attachment

PH-ACC-259
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8.196

PROGRESS REPORT NO.1

IN VITRO CONSERVATION AND PROPAGATION OF THREE
ECONOMIC SPECIES OF RATTANS

A RESEARCH PROJECT

Grant No. 936-5542-G-00-8039-00

Submitted by

Mr. Pornchai Chuthamas

Project Leader

The Royal Chitralada Projects

Bureau of The Royal Household

Chitralada Palace

Bangkok 10300

Rec'd in SCI: JAN 4 1989

PROJECT PROFILE

Country : Thailand

Grant No. : 936-5542-G-00-8039-00

Program : Program on Science and Technology
Cooperation

Project Title : In Vitro Conservation and Propagation
of Three Economic Species of Rattans

Project leader : Mr.Pornchai Chuthamas M.sc.(Horticulture)

Organization : The Royal Chitralada Projects
Bureau of The Royal Household

Co-investigators : Assistant Prof.Pranom Prutpongse M.Sc.
(Horticulture)
Associate Prof.Dr.Isara Vongkalung Ph.D.
(Forestry)
Dr.Sureeya Tantiwivat Ph.D.(Horticulture)

Project advisors : Prof.Dr.Toshio Murashige Ph.D.

Prof.Dr.Don J. Durzan Ph.D.

Associate Prof.Dr.Paiboolya Gavinlert
vatana Ph.D. (Horticulture)

Authorized officer: Mr.Keokhwan Vajarodaya

Total Project Budget : us\$ 150,000 (Bt 3,750,000)

Project Duration : 3 years

Reporting Period : June 1 - November 30, 1988

Budget Allocation for this Period : Bt 1,396,250

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I. BACKGROUND AND INTRODUCTION

Rattans are spiny climbing palms that are found in the South East Asia and neighboring regions. The species of rattan around the world may possibly number about 600 species (Dransfield, 1979). The utilization of Rattans have been known since ancient time for numeral purposes such as weaving materials, basket wares, matting, furniture etc.

At present the demand for rattan canes has been increasing year after year and overexploitation of canes from natural forest caused the depletion of natural rattan resource, extinction may be the end result. Conservation and propagation of rattans must be viewed as urgent. One of the major constraints in embarking on rattan plantation is the INSUFFICIENT AVAILABILITY OF RATTAN SEEDS. Effort to produce enough seeds, via normal propagation techniques have not gone fast enough to meet demand. Tissue culture will be a mean to accelerate stock production on a large quantities of rattan seedling since it can be produced vegetatively and extensively for mass propagation. It is anticipated that rattan propagation through tissue culture would be powerful method to meet the increasing demand for large scale plantation.

The government of the United States of America action through the Agency for International Development (AID) is funding a research project entitled "IN VITRO CONSERVATION AND PROPAGATION OF THREE ECONOMIC SPECIES OF RATTANS" by means of a grant of US\$ 150,000 to the Royal Thai Government. The effective period of this grant is May 16, 1988 to December

31,1991. It has been agreed that The Royal Chitralada Project will administer the grant on behalf of the Department of Technical and Economic Cooperation.

II. PROJECT OBJECTIVES

The aim of this project is to meet the increasing demand for rattan from the household industry by utilizing tissue culture techniques in the conservation and mass propagation of three rattan species. The stated objectives are :

1. to develop *in vitro* conservation techniques for three economic species of rattans: Wai Nam Pung (Calamus sp.) Wai Takra Thong. (Calamus caesius Blume.) and Wai Hom (Calamus pandanosmus Furtado);

2. to develop plant tissue culture techniques for mass propagation of these three species; and

3. to collect and conserve these three species by *in vitro* culture to augment the existing germplasm collection under the Royal Rattan Development Project.

III. MATERIALS AND METHODS

A few preliminary experiments have been conducted for surface sterilization and investigated the media. Three streilizing agent (Ethanol 70%, sodium hypochorite and mercuric chloride) were used at variuos concentrations and exposure time times to sterilize shoot and root tissue from seedling of Calamus caesius. Shoots were sterilizes by immersing 20, 30, 45 min. for time of disinfection at

various concentration 1%, 1.5% and 2% sodium hypochlorite and 0.05% Tween 20 emulsifier. Roots were immersed in 0.1% and 0.5% mercuric chloride in which Tween 20 emulsifier and then rinsed three times with autoclaved distilled water.

One explant was placed in each vessel of MS nutrient medium.

Three basal media MS (Murashige and Skoog 1962) (4) Y3 (Eeuwens 1976) (2) and Woody Plant Medium (WPM) (3) were investigated for development of embryo of C. perigrinus.

The medium for callus establishment contained Murashige and Skoog 1962 basal medium and added with 1-2 mg/l BAP and 1,2,4,10 mg/l 2,4-D. All nutrient media were prepared in 4 oz. vessels, each containing 25 ml media. The pH of media was set at 5.7 prior to addition of gelrite. The vessels were capped with autoclavable plastic caps, and sterilized by autoclaving 15 min at 15 lbs/inch². All cultures were incubated at 25 ± 1° and under 16 hr. daily illumination from cool white fluorescence lamps.

IV. RESULTS AND DISCUSSION

The surface sterilization experiments tested for a suitable concentrations of sterilizing agent and suitable times. Sodium hypochlorite and mercuric chloride were effective. The percent contamination for sodium hypochlorite ranged 33.82% - 87.74%. Table 1.

Table 1. Percent contamination of explant of *C.caesius* as effected by concentration of sodium hypochlorite and disinfection times.

Concentration of sodium hypo chlorite *	Times of Disinfection (min)	Number of Samples	Number and Percent of Contamination **
1.5%	20	310	272 (87.74%)
	30	141	85 (60.28%)
	45	195	82 (42.05%)
2.0%	20	184	153 (83.15%)
	30	141	85 (60.28%)
	45	204	69 (33.82%)
2.5%	20	80	47 (58.75%)
	30	115	53 (46.09%)
	45	134	52 (39.55%)

** Both Bacteria and Fungi

Mercuric Chloride was an effective sterilizing agent for root disinfection. The percent contamination ranged from 0 - 31.82 %. (Table 2)

Table. 2. Percent contamination of root explant of *C.caesius* by concentration of mercuric chloride and disinfection times.

Concentration of mercuric chloride	Times of disinfection (min)	Number of Samples	Number and Percent of Contamination
0.1%	10	22	7 (31.82%)
	20	22	1 (4.55%)
	30	20	0 (0%)
0.5%	10	24	4 (16.67%)
	20	21	0 (0%)
	30	20	0 (0%)

Preliminary results show that all three media (MS, Y³ and WPM) are suitable for embryo of C.perigrinus develop to be plantlets (Fig.1.). The growth of embryo in vitro present a useful tool for studies of rattan and will be utilized in the future work.

Callus established from young leaf and petiole tissue of C. caesius applied on to the MS medium added with BAP and 2,4-D. Especially tissue of young leaf placed on MS medium + 2 mg/l BAP + 4 mg/l 2,4-D and petiole tissue placed on MS medium + 2 mg/l BAP + 1, 2 and 10 mg/l 2,4-D were developed callus (Fig.2, Fig.3). There was no quantitative measurements conducted on growth of calli, it was observed for the future work.

V. CONCLUSION

From a few preliminary experiments, it was too early to conclude at this moment for the best procedure for culture rattan in vitro. And the nature of this period were laboratory remodelling, appointed the staff, equipment purchasing and installation. The most difficult problem was collecting the rattan from the natural forest, especially c. pandanosmus (Wai Hom), C.sp. (Wai Num Pung) and C.caesius (Wai Takra Thong). The previous number collected was limited more are required for the experiment work.

VI. WORKPLAN FOR THE NEXT PERIOD

FROM DECEMBER 1988 TO MAY 1989

1. Technology Development

Explant from in vitro seedling

Study will concentrate on the following outline :

- evaluate the appropriate media on tissue culture of rattans;
- investigate media composition and auxin-cytokinin levels to induce callus;
- study the effect of auxin-cytokinin on callus multiplication;
- study the effect of auxin-cytokinin on induction of embryogenesis.

Seedling from embryo culture

Study will concentrate on:

- multiplication of seedling in relation to growth regulators;
- the effect of low temperature and growth retardant on the growth of seedling.

2. Germplasm collection activities

The work will be comprise

- collection clones of three economic rattans from different locations.
- planting of the In vivo collection at The Royal Chitralada Palace and Department of Forest Biology, Faculty of Forestry, Kasetsart University.

3. Research consultation

Prof. Dr. D. J. Durzan from Department of Environmental Horticulture, University of California, Davis will come to Thailand during January 22/23-29/30, 1989 or January 29/30 - February 5/6, 1989. He will give advice, make suggestion and participate in the research work at The Royal Chitralada Projects - USAID Rattans Tissue Culture Laboratory, Chitralada Palace and KU - USAID Bamboo Tissue Culture Laboratory, Department of Horticulture, Kasetsart University.

VII. REFERENCES

1. Dransfield, J. 1979. A manual of the rattans of the Malay Peninsula. Malayan Forest record No. 29. Forest Dept., Peninsular Malaysia. 270 pp.
2. Eeuwens, C. J. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palm (*Cocos nucifera*) and cultured in vitro, *Physiol. Palnt.* 36. 23-8 p.
3. Lloyd, G. and B. H. McCown. 1981. Commercially-feasible micropropagation of Mountain Laurel, *Kalmia latifolia*, Linn. by use shoot-tip culture. *Proc. Intern. Plant Prop. Soc.* 30: 421-427
4. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15: 473-497.

VIII. ANNEX

1. Pictures:



Fig. 1. Embryo development of C. perigrinus



Fig. 2. Callus from young leaf of *c.caesius*

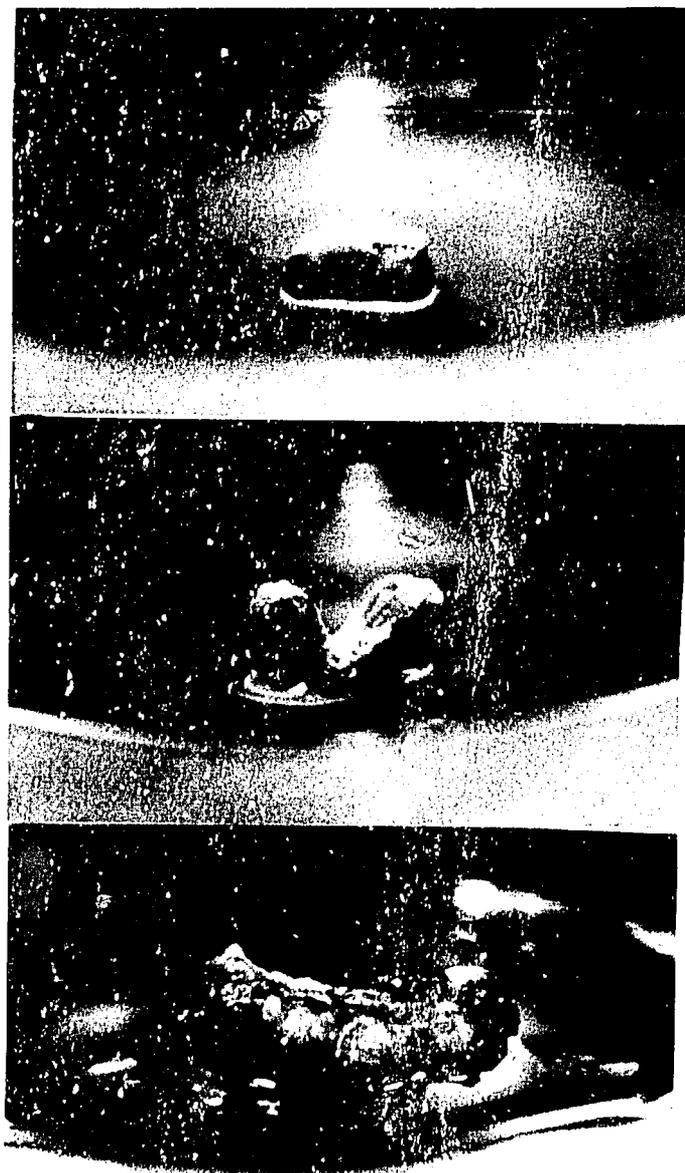
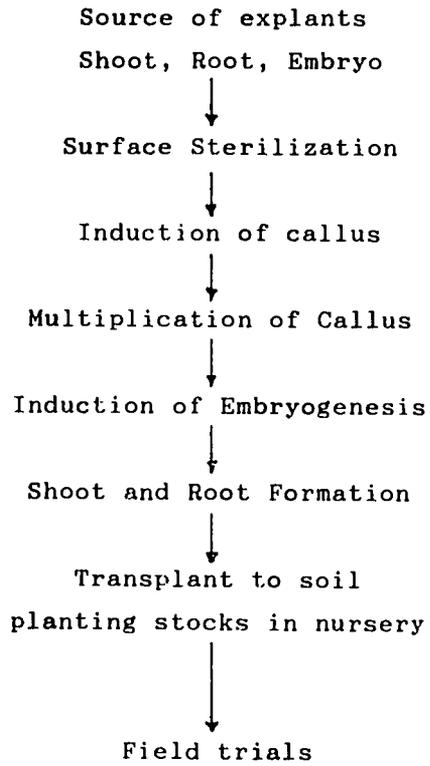


Fig.3. Callus from petiole of *C.caesius*

The schematic outline of the research programme is shown below :



Explanation of stepwise procedures

- 1). Explants of rattans will be attempted to clean culture. Seedlings and seed of rattans to be surface sterilized with various sterilizing agents.
- 2). Callus will be induced from explants of shoot, root and embryo. The effects of auxin-cytokinin levels and conditions will be examined.
- 3). Multiplication of callus will be attempted by the use of various compositions of medium and concentrations of auxin and cytokinin.
- 4). The effects of auxin, cytokinin and growth regulators on the induction of embryogenesis will be examined.
- 5). Shoot and Root Formation will be induce by various concentrations of cytokinin and/or auxin.
- 6). Transplant to soil. Plantlets will be prepared and acclimatized. This step will place emphasis on the study of suitable procedure and conditions and will explore the use of anti-transpirant film to replace the cuticle wax.
- 7). Field trials of propagated material.

2.2. Financial plan

A financial plan is shown in Table 4 and modification of financial plan is shown in Table 5. Expenditure in the first period and second period differ due to the laboratory expansion and renovation, research collaboration, equipment purchasing and installation. The other expenses are mostly operation cost.

FINANCIAL PLAN
 Grant No. 936-5542-G-00-8039-00
In vitro Conservation and Propagation of Three Economic Species of Bats

Budget Category	Year Month	1988				1989				1990				1991		TOTAL
		J J A	S O N	D J F	M A M	J J A	S O N	D J F	M A M	J J A	S O N	D J F	M A M			
Salaries/Compensation		81,250	81,250	81,250	81,250	86,250	95,250	86,250	86,250	97,500	97,500	97,500	97,500	1,060,000		
Equipments		287,500	387,500	-	-	-	-	-	-	-	-	-	-	675,000		
Materials & Supplies		67,500	113,750	90,600	90,650	96,000	60,250	78,250	78,000	110,000	52,750	81,250	75,000	1,000,000		
Travel: Local & International		32,750	48,750	16,750	43,750	16,750	43,750	18,750	18,750	68,750	13,750	18,750	18,750	375,000		
Research Collaboration & Consultation		102,500	-	150,000	-	-	-	-	150,000	-	-	-	-	492,500		
Laboratory remodelling & Maintenance		137,500	50,000	-	-	-	-	-	25,000	-	25,000	-	-	237,500		
TOTAL		715,000	681,250	340,600	215,650	201,000	190,250	183,250	358,000	276,250	200,000	197,500	191,250	3,750,000		

1 US \$ = 25 Bats

Table 4. Financial Plan

FINANCIAL PLAN
Grant No. 936-5542-G-00-8039-00

In vitro Conservation and Propagation of Three Economic Species of Eattans

Budget Category	Year	1985				1989				1990				1991	TOTAL
	Month	J J A	S O N	D J F	M A M	J J A	S O N	D J F	M A M	J J A	S O N	D J F	M A M		
Salaries/Compensation		31,250	31,250	31,250	31,250	36,250	36,250	36,250	36,250	37,500	37,500	37,500	37,500	1,060,000	
Equipments		237,500	434,000	52,000	-	-	-	-	-	-	-	-	-	774,400	
Materials & Supplies		67,500	66,350	38,600	90,650	96,000	60,250	78,250	78,000	110,000	58,750	61,250	75,000	900,600	
Travel: Local & International		18,750	46,750	18,750	43,750	18,750	43,750	18,750	18,750	66,750	18,750	18,750	18,750	375,000	
Research Collaboration & Consultation		150,500	-	150,000	-	-	-	-	150,000	-	-	-	-	402,500	
Laboratory remodeling & Maintenance		137,500	50,000	-	-	-	-	-	25,000	-	25,000	-	-	237,500	
TOTAL		715,000	691,250	340,600	215,650	201,000	190,250	183,250	352,000	276,250	200,000	137,500	151,250	3,750,000	

1 US \$ = 25 Baht

Table 5. Modification of Financial Plan

3. ACTIVITIES FROM JUNE TO NOVEMBER 1988

3.1. Laboratory expansion and renovation

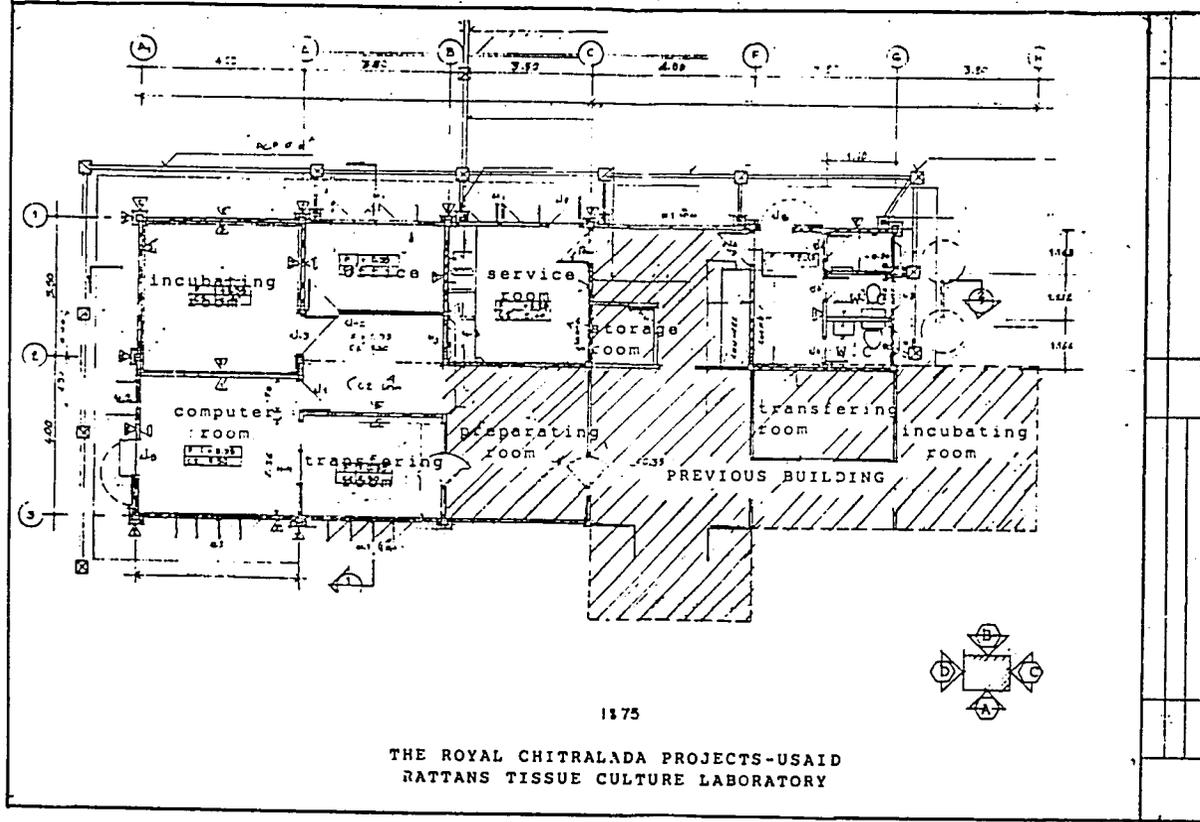
Expansion and renovation of the plant tissue culture building made a space of 124.5 m² available for the new tissue culture laboratory. The laboratory was designed in May 1988 and the works authorized in June 1988. The expansion of the building began on July 1, 1988 and was completed on August 30, 1988. The laboratory renovation was completed on September 10, 1988. The expenditure for expansion and renovation of this building came from the donation budget to H.M. The King of Thailand for The Royal Chitralada Plant Tissue Culture Project and the grants from USAID. The new facility was named "THE ROYAL CHITRALADA PROJECTS-USAID RATIANS TISSUE CULTURE LABORATORY".

3.2. Staff procurement

Two assistant researchers [Miss Piyarat Parinyapong B.Sc.(Biology), Miss Noppawan Ruangngam B.Sc.(Horticulture)] two laboratory attendants [Miss Phannee Maneeakars Certificate in accounting, Miss Ravadee Likitvong grade 12 certificate] and two nursery workers [Mr. Surat Maichan and Mr. Rungroj Butrapan] have been appointed. In addition, two co-investigator, Assistant Prof. Pranom Prutpongse and Associate Prof. Dr. Isara Vongkalung, are also involved.

Mr. Pornchai Chuthamas (Principal investigator) Dr. Sureeya Tantiwiwat (Co-investigator), Miss Manuwadee Ngaosuan B.Sc. (Horticulture) and Miss Avadapa Amartayakul B.S. (Accounting) are all Royal Thai Government officers and so have not been procured from the compensation budget.

Fig. 4. Plan of Plant Tissue Culture Laboratory, Chitralada Palace



THE ROYAL CHITRALADA PROJECTS-USAID
RATTANS TISSUE CULTURE LABORATORY

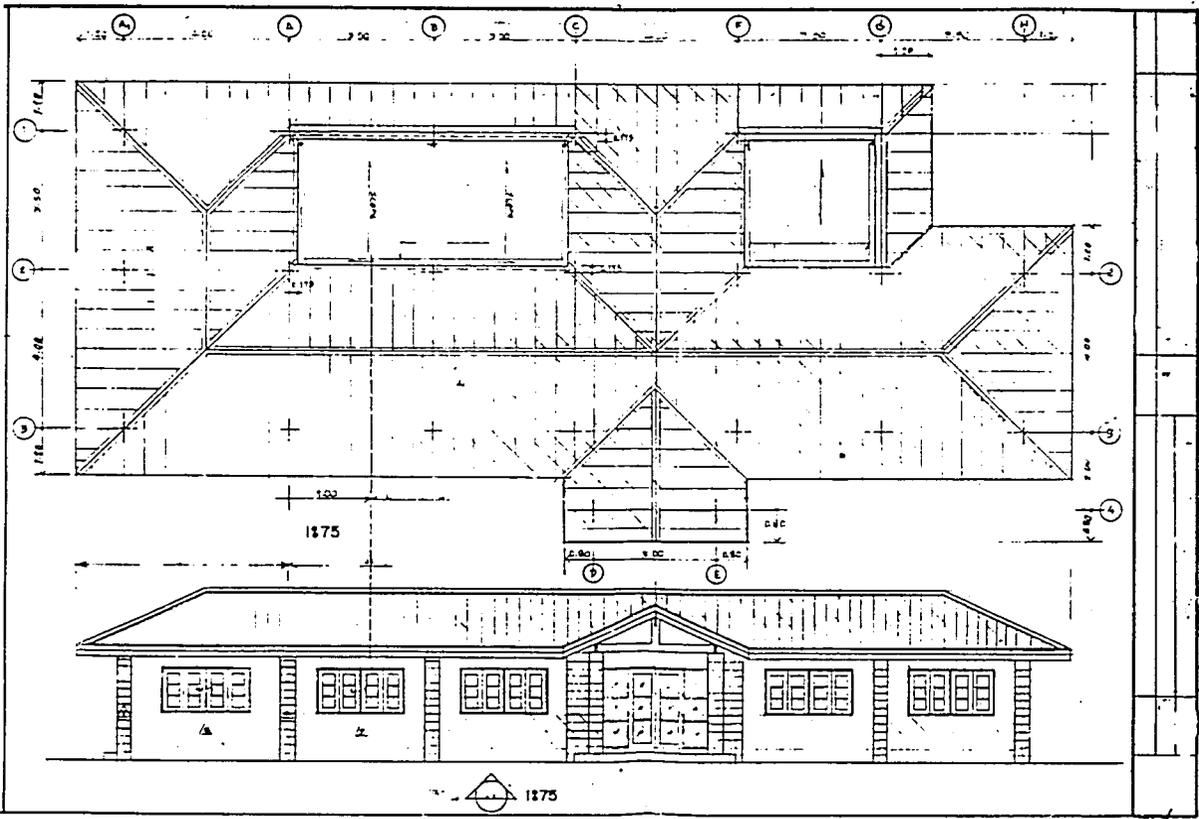
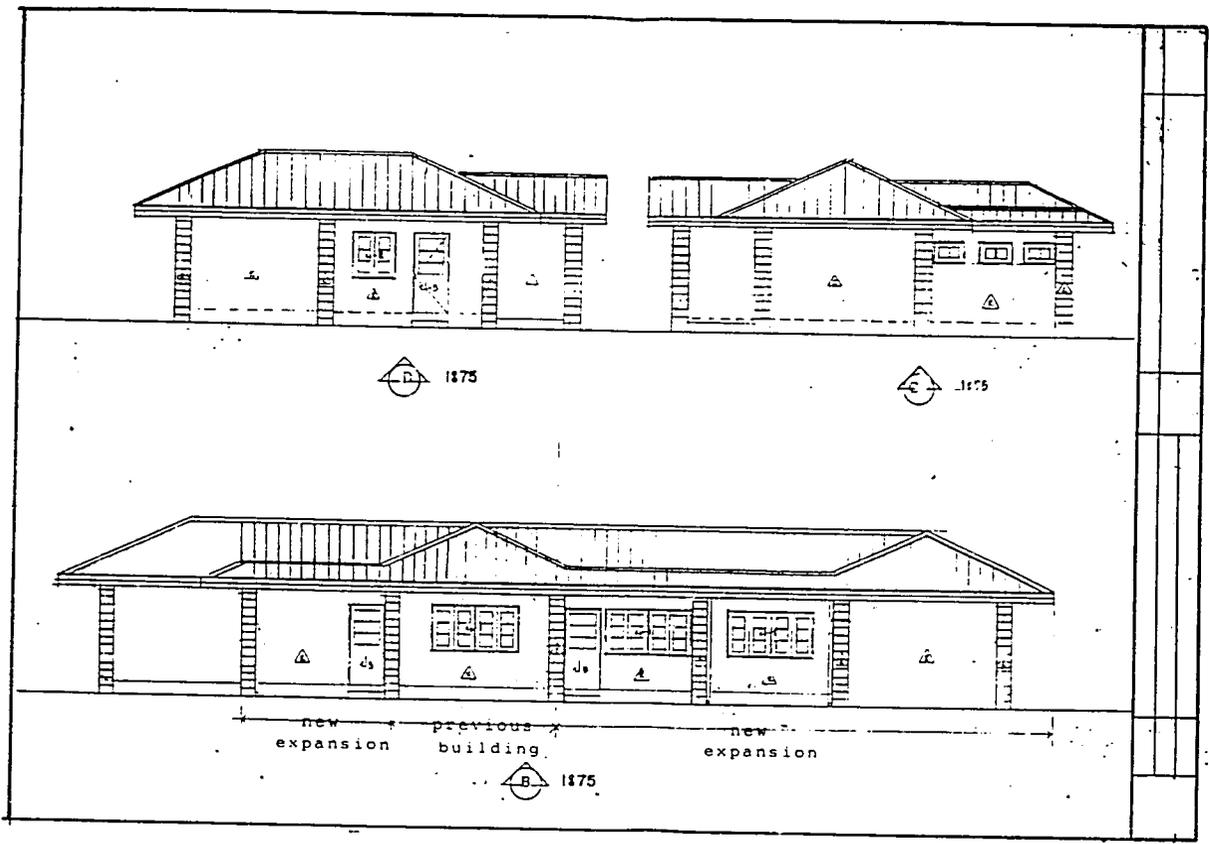


Fig. 5. Front view and Top view of Plant Tissue Culture Laboratory
Chitralada Palace

Fig. 6. Side veiw and Back veiw of Plant Tissue Culture Laboratory
Chitralada Palace.



3.3. Equipment purchasing and installation

The following equipment has been purchased and installed in "THE ROYAL CHITRALADA PROJECTS-USAID RATTANS TISSUE CULTURE LABORATORY"

- 5 air conditioners
- 1 bench top laminar flow station
- 28.8 sq.m. incubating shelves
- 1 refrigerator 18 cu/ft.
- 1 shaker
- 1 trinocular microscope

The following equipment await delivery

- 2 growth chambers 18.8 cu/ft
- 1 freezer incubator
- 1 stereozoom microscope

Chemicals and glassware have been purchased as required. Additional consummable items will be purchased as required throughout the duration of the project.

3.4. Research activities

Research activity started since August 1988 at the Royal Chitralada Palnt Tissue Culture Laboratory and after September 10, 1988 research activities were carried on at "THE ROYAL CHITRALADA PROJECTS-USAID RATTANS TISSUE CULTURE LABORATORY".

Work in progress

3.4.1. Rattan Survey and Collection.

A field trip was made to Chumporn province to search for germplasm and research material, especially Calamus pandanosmus (Wai Hom). Five days were spent searching for this rattan species, which is now very difficult to find in the natural forest because it quite sparse and only persist in the deep forest. C.pandanosmus is a species offering high potential for handicraft and household potential used and was harvested heavily in the past.

However it is quite fortunate that 20 plants were found. They were planted in plastic bag and brought to Bangkok, 2 plants were cultured immediately, 18 plants were potted and kept in the nursery until they will be well established at Chitralada Palace, and Department of Forest-Biology and Department of Horticulture, Kasetsart University. Since number collected was limited more are required for experimental purposes, the next field trip will be made verysoon in order to collect more materials.

The second team of researchers travelled to survey and collect material in Trang province. They spent three days in searching for Calamus caesius and other species in Khao Chong Nature and Wildlife Study Centre, Muang District. It proved difficult to find C.caesius but rattan fruits of C.perigrinus were collected to serve as germplasm for tissue culture experiments.

3.4.2 Tissue Culture experiments

A few preliminary experiments have been conducted.

3.5. Research observation and collaboration

Mr.Pornchai Chuthamas (Principal Investigator) travelled to the United States of America in the first period of the project during July 17- August 20,1988 and attended the Agricultural Application in Plant Tissue Culture, Summer Session Course at the Department of Botany and Plant Science University of California Riverside, from July 18- August 5, 1988. This course was taught by Prof. Dr.Toshio Murashige (Project Advisor). Afterwards he visited the laboratory of Prof. Don J.Durzan (Project advisor) at Department of Environmental Horticulture, University of California, Davis during August 9-13,1988. He had an opportunity to learn some techniques in this laboratory and discuss the rattan research programme with Prof.Durzan. He also met Prof.D.W.Burger and Prof.A.Dandekar who also gave suggestions about the project. He visited Dr.Janet Rice at the office in AID buliding, Washington D.C. and visited USDA Agriucultural Research Centre at Beltsville, Maryland during August 15-18 1988. In the Agricultural Research Centre he visited the laboratories of Dr.L.D.Owens, Dr.F. A.Hammerschlag and Dr.R.J.Grisbach to see some new advanced techniques in plant tissue culture.

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