6 TH AND 7 TH INTERIM REPORTS

FOR

IN VITRO CONSERVATION AND GERMPLASM EXCHANGE OF BAMBOO

HORTICULTURE DEPARTMENT
FACULTY OF AGRICULTURE
KASETSART UNIVERSITY
BANGKHEN
BANGKOK 10903
THAILAND
TEL ¦ 5791951

US. AGENCY FOR INTERNATIONAL DEVELOPMENT
USIAD, THAILAND
37 SOI SOMPRASONG 3
BANGKOK 10400
THAILAND

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INVESTIGATORS

1. Prof. Dr. BUNJERD KATIKARN Ph.D.	Project Advisor
2. Associate Prof. Dr. PAIBOOLYA GAVINLERTVATANA Ph.D	Principal Investigator
3. Assistant Prof. SANUN KHUMLERT B.Sc.	Co-investigator
4. Assistant Prof. PRANOM PRUTPONGSE M.Sc.	Co-investigator
5. Dr. UTAI JARANASRI Ph.D.	Co-investigator
6. Miss. THANTAWAN POONSAWAT M.Sc.	Technician
7. Mr.TAVATCHAI VATHANAVALUM M.Sc.	Technician
8. Miss. KANYARATT SUPAIBOONWATANA B.Sc.	Trainee
9. Mr. VIBOON SUKCHAROEN	Grade 12 Lab attendant

ORGANIZATION

HORTICULTURE DEPARTMENT

FACULTY OF AGRICULTURE

KASETSART UNIVERSITY

BANGKOK, THAILAND 10903

TEL. 579-0113 Ext. 330 or

579-1951

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I. INTRODUCTION

The Government of the United State of America, acting through the Agency for International Development (AID) granted a research project entitled; "IN VITRO CONSERVATION AND GERMPLASM EXCHANGE OF BAMBOO" an amount of US $ 150,000 to the Royal Thai Government. The effective date of this grant started from June 10, 1985 to October 31, 1987. It is agreed that Kasetsart University will act on behalf of the Department of Technical and Economic Cooperation concerning the administration of this grant, and implementing the research Project for the Royal Thai Government.

The ultimate goal is to develop new technology using tissue culture for bamboo conservation and propagation. Specific research objectives include:

1). *Development of in vitro conservation techniques;*

2). *Collection and conservation of bamboo varieties grown in Thailand and Sri Lanka using tissue culture techniques:*

3). *Development of tissue culture techniques for mass propagation; and*

4). *Development of techniques for international bamboo germplasm exchange using tissue culture.*

The first interim report covered the activities from June to December, 1985. Contents included laboratory renovation, hiring of technical staff, equipment purchasing and installation and initiation of research activities. The second interim report covered from January to June, 1986 . Main emphasis forcused on research activities and germplasm collection and administrative work. The third interim report covered from June to December, 1986 . This

report covers the 6th and the 7th interim report from January, 1988 to June, 1988 and from July, 1988 to December, 1988 the two interim reports are combined and presented to the USAID office here with

II ACTIVITIES

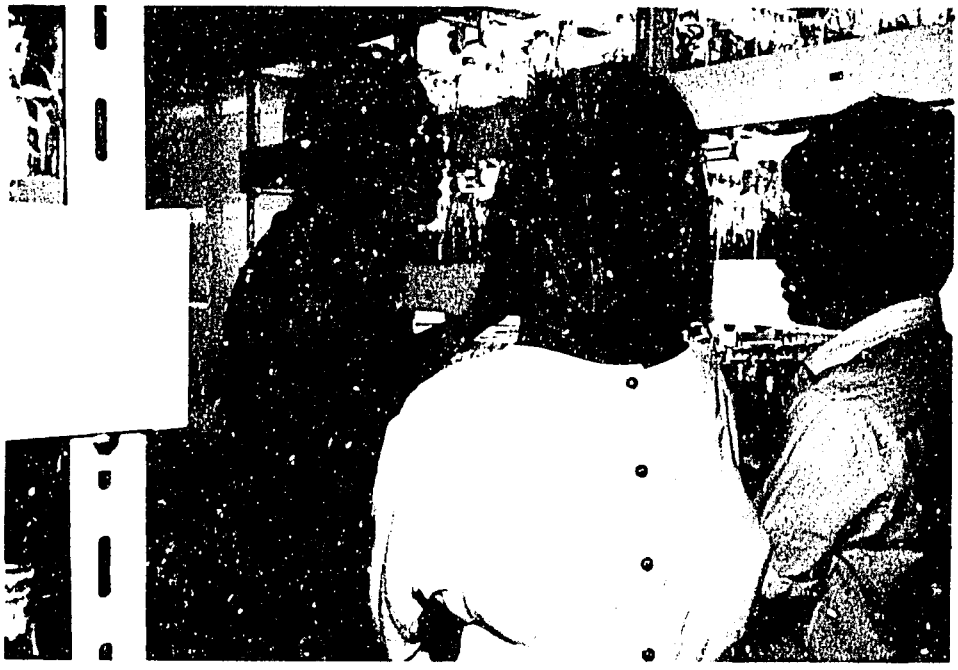
1. Prominent visitor

KU-USAID Bamboo Tissue Culture Laboratory at Kasetsart University had an apportunity to welcome Professor Dr.Toshio Murashige, a prominent tissue culturist, from University of California,Reverside, USA during April 15-20, 1989 and gave a very useful advise to the project. Picture 1 and 2 showed Professor Dr.Toshio Murashige was investigating and discussing with Bamboo researchers at the Bamboo Tissue Culture Laboratory premise. During this period, several other prominent scientists from Foreign countries had also visited Bamboo Tissue Culture Laboratory, as following :-

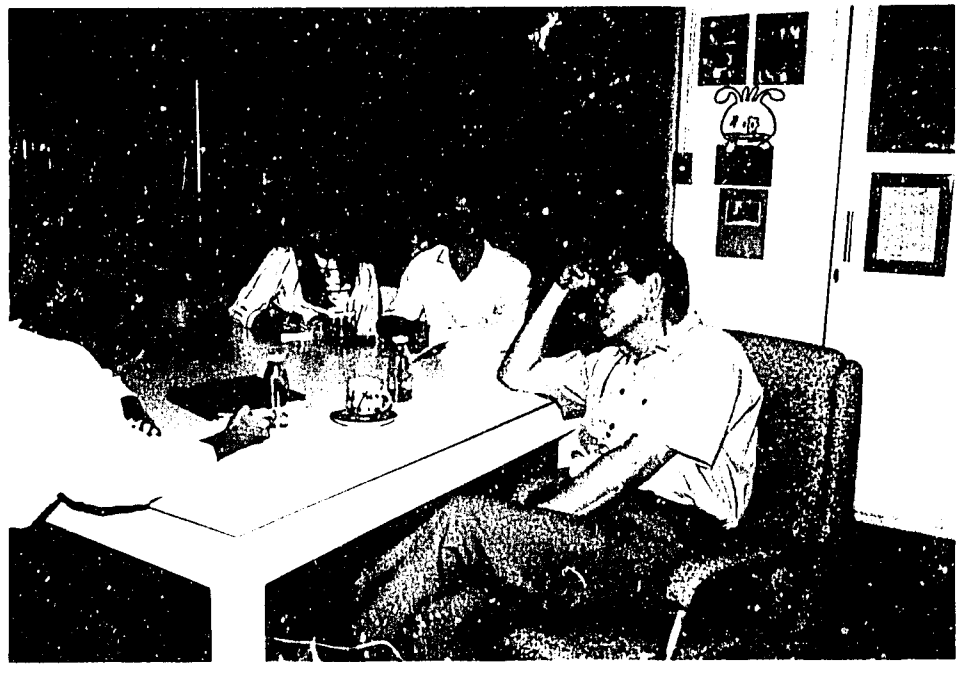
Dr.David Thurston,	Cornell University Ithaca, NY. USA.
Dr.Norman Nicholson	AID/W USA.
Dr.Gebhard Befler	University of Hotenhein
Dr.Hubert Sterba	University für Bodenkultur Wien.
Dr.Walter Larvher	University of Innobruck.
Dr.Jenet Rice	USAID-Washington
Prof.Dr.Eizo Maeda	Nagoya University Japan
Dr.Masato Ikegami	Tokyo University Japan
Dr.Lee Medema	Forest Fuel wood Rescarch Development Winrock International USA.

Dr.Norman Darrey	the University of Sydney, Australia
Dr.Hitoshi Kimuna	President of Kagawa University Japan
Dr.Dëborah Dawson	U.S. Senate Appropriations committe, Agriculture Subcommitte Washington D.C.
Dr.Duane Acker	U.S. AID Washington U.S.A.
Dr.John Temouth	University of Queensland St, Lucia Queensland. Australia
Carl-otto ottosen	Institute of Glass house crops, Denmark
Dr.Ian G. Morison	Senior Research officer U./Inst. USA.
Dr. M.V.Rao	Special Director General New Deli. India
Dr.Ashley Morton	Research Director University of Southampton , England
Dr.Li ZaiLowg	Zhejiang Agri Univ. China

etc.



Picture 1 : Professor Dr. T.Murashige investigating at Bamboo Culture Tissue Culture Laboratory Kasetsart University, Bangkok on April 20, 1988

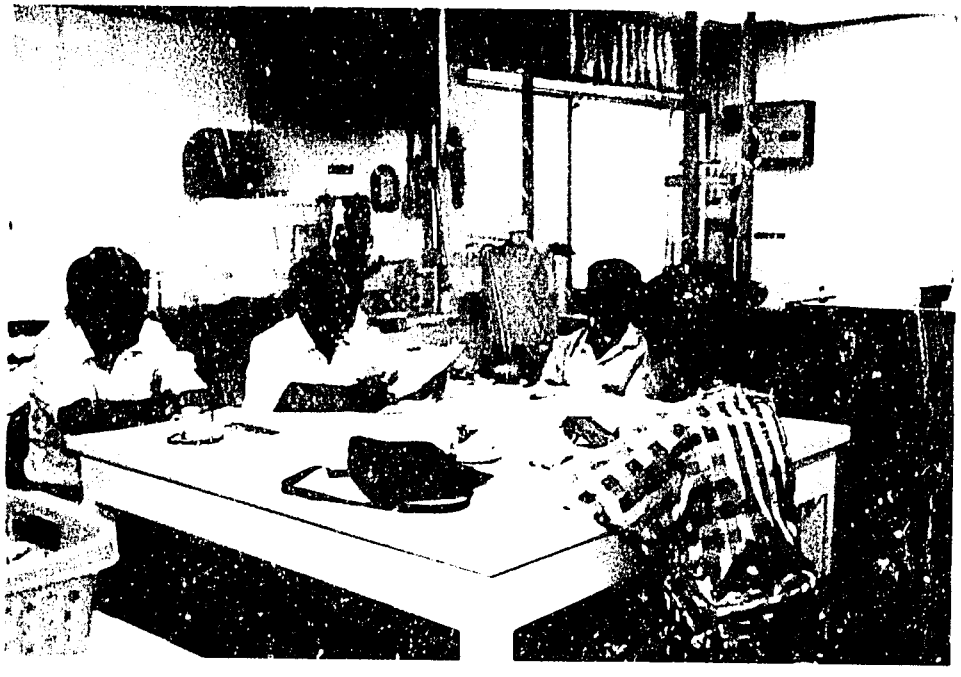


Picture 2 : Prof. Dr.T.Murashige discussed with researchers at Bamboo Tissue Culture Laboratory, Kasetsart University, Bangkok on April 20, 1988

2. Training

KU-USAID Bamboo Tissue Culture Laboratory had organised 1 month training for three Sri Lankan counterparts, starting from February, 3 to March, 4 1988. Training programmes were included theoretical and, practical work under the Laminar Flow Hoods and field trips for Bamboo collection. It was a very successful training programmes. Sri Lankan researchers had been a quainted with project concepts, practical experienced in bamboo tissue culture. Several bamboo cultures were given to trainees to bring back for their laboratory in order to continue their research activities.

Picture 3 and 4 showed parts of training activities in the laboratory at Kasetsart University



Picture 3 : Training for three Sri Lankan researchers on Bamboo Tissue Culture course at Kasetsart University Bangkok from February 3 to March 4, 1988

Picture 4 : Practical training for Sri Lankan participant in Bamboo Tissue Culture at Kasetsart University, Bangkok from February 3 to March 4, 1988

3. Conference

Researcher from Bamboo Tissue Culture Laboratory, Kasetsart University who has been supported by USAID attended and presented paper entitle, "In Vitro conservation and germplasm exchange of Bamboo" at Washington D.C. on July, 1988.

III RESEARCH ACTIVITIES

Similarly to the previous reports (4th and 5th interim reports) research activities during January - December, 1988 focused on the extension of tissue culture research on several other bamboo species, germplasm collection and development of eryopreservation and field trial
The other following research activities are :-

1. Research on culture propagation via axillary buds enhancement :

   1.1 Dendrocalamus

Explants produced multiple shoots on MS medium containing BA at 2 mg/l + NAA at 1 mg/l or MS medium supplement with BA at 5 mg/l. Attempt was made to induce root of these shoots. Several experiments including the investigation of auxins at different levels, and at various types of auxin ie. NAA, IBA and 2,4-D were conducted.
It was found that medium consisted of MS basal salts supplemented

with NAA at 0.5 mg/l was suitable for shoots to produce roots. Eight percent of shoots producing root were acheived.

1.2 Thyrsostachys oliveri Gamble.

Explants produced multiple shoots on MS medium containing BA at 2 mg/l NAA at 1 mg/l. Multiplication rate was around 5 times for every 1-1$\frac{1}{2}$ months. Experiments conducted for rooting were successful. Up to 90 percent rooting was obtained on shoots which were cultured on medium containing NAA at 0.5 mg/l.

1.3 Cephalostachym pergracile Munro.

Similarly to Dendrocalamus sp. and Thyrsostachys oliveri, multiple shoots were obtained on the explants which were cultured a MS medium containing BA at 2 mg/l and NAA at 1 mg/l. In a similar medium composition for multiple shoots, formation, root developed simultaneously. In order to avoid rooting in multiple shoots. Sub-culture frequently for every month was necessary. In contrary, left cultures in the medium for a longer period of time if root formation was desired (Table 1).

Table 1. Simultaneous rooting in multiple shoots cultures of *Cephalostachym pergracile* Munro. which were root subcultured for 1 to 4 months

Times (months)	percent of root formation
1	0
2	10
3	30
4	100

1.4 *Shizostachym* sp.

Explants were generally not multiplied into multiple shoots. Explants grew upto 1-2 leaves stages, then died. In same cases, shoots survived but did not multiply. Attempts were made to root them but without conclusive results.

1.5 *Bambusa multiplex nana*

Explants produced multiple shoots when they were cultured on MS basal medium supplemented with BA at 5 or 10 mg/l. or with 2iP at 20 mg/l. Experiments were conducted to induce roots on these shoots. It was found that shoots produced roots (75%) when they were cultured or a medium containing NAA at 10 mg/l.

1.6 Bambusa multiplex

Multiple shoots were obtained when explants were cultured on a MS basal mudium containing BA at 2 mg/l anu NAA at 1 mg/l or BA at 10 mg/l. When shoots were seperated and cultured on a MS medium containing BA at 2 mg/l and NAA at 1 mg/l 75% or on a medium containing BA at 5 mg/l formed roots simultaneously.

1.7 Schizostachym aciculare Gamble.

Multiple shoots were produced when explants were cultured on a medium containing BA at 5 or 10 mg/l. However, shoots formation was not prolific. Only 1-2 shoots were formed in a period of 3 months. Twenty five percents explants were roots simultaneously.

2. Research on tissue culture propagation via callus :

A. Callus Induction

A 1. Bambusa vulgaris. Leaf explants produced callus when they were cultured on a MS medium containing 2,4-D at 3 mg/l and sucrose at 2%. If they did, it was a small amount of callus formation.

A 2. Thyrsostachys simensis. Leaf explants produced callus on a medium containing 2,4-D at 3 mg/l (40%)

A 3. Phylostachys pubescens. Leaf explants produced callus up to 90% of the explants when they were cultured or a medium containing 2,4-D at 3 or 6 mg/l 2,4-D at above 6 mg/l inhibited callus fermation.

A 4. Bambusa glaurescens. Leaf explants produced callus at about 50% of the when they were cultured on a medium containing 2,4-D at 3 mg/l. However, callus was easily brown and dried. Small amount of callus was formed on the explants.

A 5. Chimonobambusa quadrangularis. Leaf explants produced callus when they were cultured on a medium containing 2,4-D at 1,3 and 6 mg/l. The higher the concentration of 2,4-D, the higher the percentages of callus formation. For instance, callus formation at 80% when tissue were cultured on 2,4-D at 6 mg/l medium compared to 50% callus or 2,4-D at 3 mg/l medium.

Table 2 Percent callus formation of various kinds of Bamboo on different types of medium

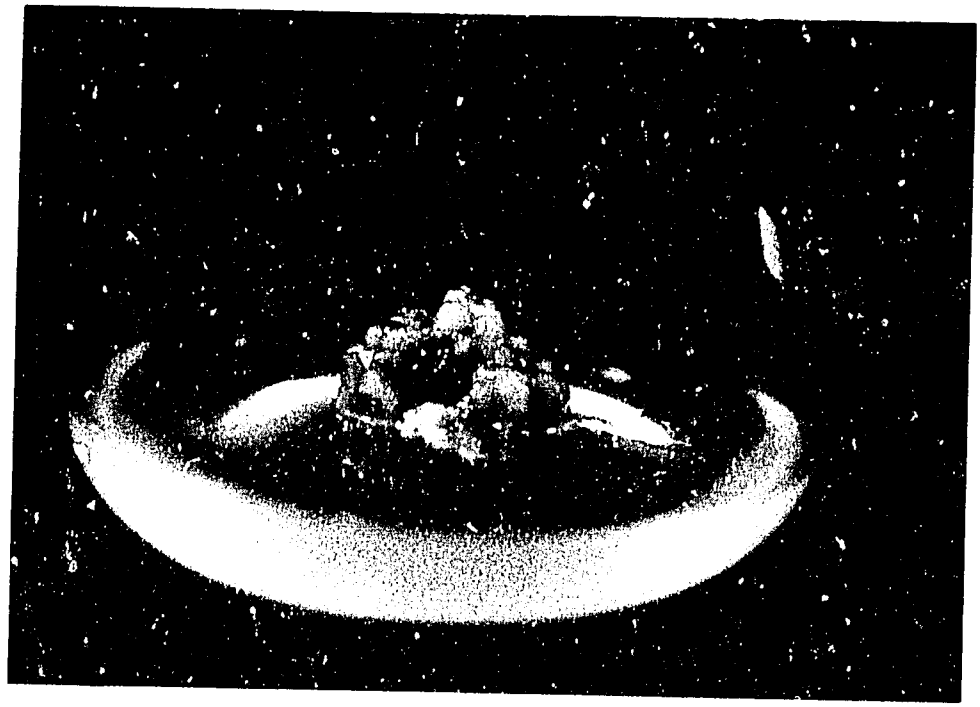
Species	Medium Types						
	M_1	M_3	M	M_9	M_{18}	W_3	W
Bambusa vulgaris	-	85	-	-	-	0	0
Thyrsostachys simensis	-	40	-				
Chimnobambusa quadrangularis	50	72	80	-	-	-	-
Phylostachys pubesens	70	80	90	40	20	-	-
Bambusa glaurescens	-	55	-	-	-	-	-

- = no treatment was conducts.

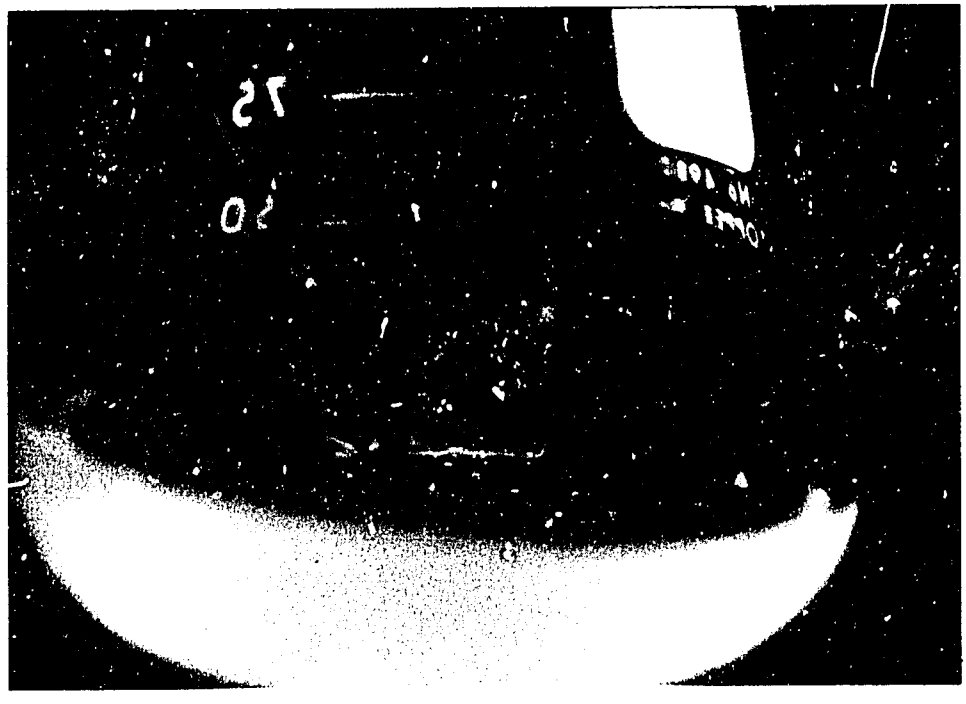
B. Embryoid Induction

B 1. Dendrocalamus membranaceus Munro. Callus was formed on leaf explants which were cultured on a medium containing 2,4-D at 5 mg/l. Callus was then multiplied on a medium containing 2,4-D at 3 mg/l. Callus multiplied rapidly and friable (Picture 5) Experiments were conducted to induce embryoid formation in these calli. Several medium formular were used. After 2 months. Callus which was cultured on a reduced concentration of 2,4-D (at 1 and 2 mg/l) has changed its morphological structure. It formed a globular stucture (Picture 6). When the globular callus was transfered to a medium containing 2,4-D at 0.1 mg/l many embryoid like structure formed (Picture 7).

Picture 5 : Callus with friable structure of *D. membranaceus* on a medium containing 2,4-D at 3 mg/l



Picture 6 : Callus with globular structure of D. membranaceus on a medium containing 2,4-D at 1 or 2 mg/l



Picture 7 : Embryoid-like structure formation on D. membranaceus callus which was cultured on a medium containing 2,4-D at 0 or 0.1 mg/l

3. Research on transplanting of tissue cultured plantlets to the soil.

Experiments were conducted to transplant tissue cultured plantlets of Dendrocalamus membranaceus, Thyrsostachys oliveri and Dendrocalamus tulda simensis to the soil. It was successful. A 100 % survive was a cheived. Tissue cultured plants have been further planted in the field.

Table 3 showed the stages of Bamboo tissue culture development in this report period, January-December, 1988.

Table 3 : Status of tissue culture research on various kinds of bamboo.

Species	Stages of tissue culture development				
	Multiple shoot	Rooting	Plantlet	Trans-planting	Cryopre-servation
Thyrsostachys oliveri	✓	✓	✓	✓	*
Dendrocalamus sp.	✓	✓	✓	✓	*
Cephalostachym pergracile	✓	✓	✓	✓	
Dendrocalamus tulda	✓	✓	✓	✓	
Pleioblastus fortunee	✓	✓	✓	✓	
Bambusa arundinacea	✓	✓	✓	✓	
Bambusa multiplex nana	–	–	✓	✓	
Bambusa multiplex	–	–	✓	✓	
Schizostachym aciculare	–	–	✓	✓	
Dendrocalamus asper	–	–	✓	✓	
Schizostachyum brachycladium	–	–	✓	–	

Table 3 (cont'd)

Species	Stages of tissue culture development				
	Multiple shoot	Rooting	Plantlet	Trans-planting	Cryopre-servation
Arundinaria suberecta	–	–	✓	✓	
Bambusa vulgaris	–	–	✓		
Bambusa ventricosa	–	–	✓		
Bambusa glaucescens f. solida	–	–	✓	✓	
Dendrocalamus nutans	✓	✓	✓	✓	
Semiarundinaria fastuasa	✓	✓	✓	✓	
Dendrocalamus strictus	*				
Sasaella suwekoana	–		✓		
Hibamobambusa tramguillans	–		✓		
Gigantochloa hosseusii	✓	✓	✓	✓	
Dendrocalamus sp.	✓	*			
Thyrsostachys simensis nana	✓	*			

Table 3 (cont'd)

Species	Stages of tissue culture development				
	Multiple shoot	Rooting	Plantlet	Trans-planting	Cryopre-servation
Bambusa glaucescens	✓	*			
Gigantochloa ligulata	✓	✓	✓	✓	
Cephalostachym virgatum	*				
Dinochloa scandens	*				
Arundinaria pusilla	*				
Bambusa nutans	*				
Bambusa nana	*				
Gigantochloa apus	*				

* = Research is in progress

\- = no multiple shoots formation

4. Germplasm Conservation

4.1 Low temperature conservation :

Experiments were conducted to evaluate the effect of temperature on bamboo cultures conservation. Table 4 showed the responses of cultures when they were incubated at 10 °c for 1 to 3 months.

Table 4 Responses of various bamboo cultures under 10 °c incubation for 1 to 3 months.

Species	Times of incubation (months)	
	1	3
Dendrocalamus tulda Simensis	-leaves remained green, no root and shoot production -no growth	-leaves turned brown (100%)
Arundinaria suberecta	-leaves remained green no root or shoot formation -no growth	-Most of the leaves turned brown (75%)
Bambusa vulgaris	-leaves remained green, no root or shoot formation -no growth	-All leaves turned brown (100%)
Control (25 °c)	-leaves remained green multiple shoots fermation	-multiple shoot fermation, still green

4.2 Conservation in liquid nitrogen(-196 c) Several experiments were contucted to evaluate freezing programmes, types of cryopreservative agents ie. DMSO and thawing technique. After pre-treatment with DMSO, slow freezing at -0.5 °c/min to 0.9 °c/min to -40 °c or -70 °c, then kept in liquid nitrogen for 1 hr. and subsequently subjected to a fast thawing at 37 °c, most of tissues remained green. However, when they were cultured on a multiplied medium and incubated at 18 °c or 27 °c, tissues were not recovered, but turned brown in 7 days.

IV Germplasm collection

In addition to germplasm collection from previously report on the 4th and 5th interim report , Table 6 showed the activities of germplasm collection during January to December 1988, Table 5 showed up to date availability of bamboo germplasm in vitro at USAID-KU Bamboo Tissue Culture Laboratory.

Table 5 Availability of Bamboo germplasm *in vitro* at USAID-KU Bamboo Tissue Culture Laboratory

Bamboo species	Type of collected tissue in vitro
Thyrsostachys oliveri	multiple shoot, plantlet
Dendrocalmus sp.	multiple shoot, plantlet
Cephalostachym pergracile	multiple shoot, plantlet
Dendrocalamus tulda	multiple shoot, plantlet
Pleioblastus fortunee	multiple shoot, plantlet
Bambusa arundinacea	multiple shoot, plantlet
Dendrocalamus nutans	multiple shoot, plantlet
Semiarundinaria fastuasa	multiple shoot, plantlet
Gigantochloa hosseusii	multiple shoot, plantlet
Gigantochloa ligulata	multiple shoot, plantlet
Bambusa multiplex nana	plantlet
Bambusa multiplex	plantlet
Schizostachym aciculare	plantlet
Dendrocalamus asper	plantlet
Schizostachyum brachycladium	plantlet
Arundinaria suberecta	plantlet
Bambusa vulgaris	plantlet
Bambusa ventricosa	plantlet
Bambusa glaucesoens f. solida	plantlet
Sasaella suwekoana	plantlet
Hibamobambusa tranquillans	plantlet
Dendrocalamus sp.	multiple shoot
Thyrsostachys simensis nana	multiple shoot
Bambusa glaucescens	multiple shoot

Table 5 (cont'd)

Bamboo	Type of collected tissue in vitro
Thyrsostachys simensis	multiple shoot
Dendrocalamus membranaceus	multiple shoot, callus
Gigantochloa densa	multiple shoot
Dendrocalamus brandisii	multiple shoot
Dendrocalamus giganteus	multiple shoot
Gigantochloa accter	bud
Dendrocalamus strictus	bud
Cephalostachym virgatum	bud
Dinochloa scandens	bud
Arundinaria pusilla	bud
Bambusa nana	bud
Bambusa nutans	bud
Gigantochloa apus	bud
Bambusa sp.	bud
Bambusa beecheyama pubescens	multiple shoot
Bambusa burmanica	callus
Dendrocalamus asper clone 1	callus, multiple shoot
Dendrocalamus latiflorus	bud, multiple shoot, callus
Phyllostachys pubescens	multiple shoot

Table 6 Status of Bamboo Collection and Cultures. (January-December 1988.)

Date of collection	Species	Clones	Places of collection	Forms of collection		Comments[1/]
				branches	shoots	
January 13, 1988.	*Gigantochloa densa*	-	Kanchanuburi	/		D
January 21, 1988.	*Dendrocalamus latiflorus*	-	Prajinburi	/		P
	Dinochloa scandens	-	Nakornrachasima	/		G
February 8, 1988.	*Bambusa burmanica*	Van-Lei	Chiangmai	/		D
	B. multiplex	Saechuan-Tong		/		G
	B. nana	Kanrom		/		D
	B. Longispatha	-		/		D
	Dendrocalamus strictus	Sang-Yen		/		D
	Sasa fortunae	-		/		H
February 26, 1988.	*Bambusa polymorpha*	-	Chaingmai	/		H,D
	Chimonobambusa quadrangularis	Doi-Pui		/		C
	Dendrocalamus strictus	Sang-Mon		/		D
	Gigantochloa hosseusii	-		/		G
	Oxytenanthera albociliata	-		/		D

Table 6 (Cont'd.)

Date of collection	Species	Clones	Places of collection	Forms of collection		Comments[1/]
				branches	shoots	
February 27, 1988.	*Bambusa beecheyama var. pubescens*	Taiwan	Chaingmai	/	/	C
	B. dolichoclada	-		/		D
	B. oldhamii	-		/		G
	Dendrocalamus latiflorus	-		/		H
	Phyllostachys pubescens	Chaina		/	/	C,P
April 14, 1988.	*Dendrocalamus asper*	Tong-Dam	Bangkok		/	C,P
May 10, 1988.	*Dendrocalamus asper*	Tong-Dam	Bangkok	/	/	C,D
	D. asper	Tong-Keaw			/	C
May 13, 1988.	*Bambusa glaucescens*	-	Bangkok		/	C
May 16, 1988.	*Bambusa burmanica*	Tong-Papoom	Kanchana-buri		/	C
May 17, 1988.	*Dendrocalamus latiflorus*	1	Patoomtanee	/	/	G,C
	D. latiflorus	2		/		G,P

Table 6 (Cont'd)

Date of collection	Species	Clones	Places of collection	Forms of collection		Comments[1/]
				branches	shoots	
May 20, 1988.	*Bambusa burmanica*	VanHouy-Luk	Chaingmai	/		P
	B. dolichoclada	Taiwan		/		D
	B. edulis	Taiwan		/		D
	B. oldhamii	Taiwan		/		G
	Dendrocalamus latiflorus	Taiwan		/		H,D
	Phyllostachys pubescens	China		/		G,P
June 25, 1988.	*Bambusa beecheyama var. pubescens*	-	Chaingmai	/	/	C
	B. oldhamii	Sanmakogvan		/		G
	B. oldhamii	Taiwan		/	/	G,C
	Dendrocalamus latiflorus	Taiwan		/		D,H
	Phyllostachys pubescens	Japan		/	/	G,C

Table 6 (Cont'd.)

Date of collection	Species	Clones	Places of collection	Forms of collection		Comments 1/
				branches	shoots	
July 17, 1988.	*Dendrocalamus asper*	Tong-Dam	Bangkok		/	D
July 20, 1988.	*D. asper*	Tong-Dam	Bangkok	/	/	G,Do
July 21, 1988.	*D. asper*	Tong-Van	Prajinburi		/	
	D. latiflorus	-		/		D
July 25, 1988.	*D. latiflorus*	-	Pratoomtanee	/	/	C,G
July 30, 1988.	*Dendrocalamus brandisii*	Bong-Yai			/	D
	Gigantochloa densa	-			/	D,H
	Thyrsostachys siamensis	Rauk-Daeng	Kanchanaburi		/	C
August 5, 1988.	*Dendrocalamus asper*	Tong-Van	Kanchanaburi		/	P,C
	D. membranaceus	-			/	G
August 24, 1988.	*D. asper*	Tong-Dam	Bangkok	/		G,P
September 5, 1988.	*B. longispiculatha*	Tong-Dam	Bangkok	/		P
September 6, 1988.	*Thyrsostachys siamensis*	Tong-Dam	Bangkok	/		G

Table 6 (Cont'd.)

Date of collection	Species	Clones	Places of collection	Forms of collection		Comments 1/
				branches	shoots	
September 7, 1988.	*Dendrocalamus asper*	Tong-Keaw	Bangkok	/	/	G,C
September 14, 1988.	*Dendrocalamus*	Tong-Dam	Bangkok	/	/	D
	D. asper	Tong-Keaw		/	/	P,G
September 28, 1988.	*Dendrocalamus brandisii*	Bong-Yai	Kanchanaburi		/	D
October 3, 1988.	*Gigantochloa densa*	-	Kanchanaburi		/	D
October 12, 1988.	*Bambusa longispiculatha*	-	Prajinburi	(inflorescens)		-
	B. langispiculatha			/		H
	B. nana			/	/	P
	Dendrocalamus asper			/		G,Do
	D. latiflorus				/	P
November 1, 1988.	*Dendrocalamus asper*	Tong-Dam	Bangkok	/		G
November 8, 1988.	*Bambusa burmanica*	Bong-Yai	-		(seed)	G
	Dendrocalamus brandisii	Bong-Van	Lei		(seed)	G

Table 6 (cont'd)

Date of Collection	Species	Clones	Places of collection	Forms of collection		Comments[1]
				branches	shoots	
November 10,1988.	*Bambusa arundinaceae*	-	Prantoomtanee		/	C
	Dendrocalamus latiflorus	-		/		D
	Phyllostachys aurea	Thong-Yai		/	/	G,C
November 10,1988.	*Bambusa burmanica*	Bong-Van		/		C
	Bambusa longispiculata	-		/	/	D
	Dendrocalamus asper	Tong-Dam	Prajinburi	/	/	G,C
		Tong-Keaw		/	/	G,C
		Tong-Moa		/	/	D
December 8,1988.	*Bambusa sp.*	Petchaboon	Petchaboon	/		G,P
	Bambusa sp.	Pai-Kama		/		G
	Bambusa sp.	Gai-Reay		/		G,Do
	Bambusa sp.	Pai-Ngeang			/	G
	Bambusa multiplex	-			/	G
	Dendrocalamus giganteus	Pai-Paw			/	G
	Oxytenanthera albociliata	Pai-Kaey			/	P,G
	Phyllostachys sp.	Taiwan			/	Do

V. Future Work Plan (January-April, 1989)

1. Technology Development

   1.1 Stem-bud segments system

   - Continue experiments on rooting and transplanting of bamboo into the soil.
   - Conservation of tissue cultured plants which have been planted in the field.
   - Continue to develop cryopreservation technique

   1.2 Callus system

   - Continue to conduct experiment on embryogenesis and plantlet regeneration

2. Germplasm collection

3. Report :

   Final report will be prepared to USAID office.