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3rd INTERIM REPORT

FOR

IN VITRO CONSERVATION AND GERMPLASM EXCHANGE OF BAMBOO

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I. INTRODUCTION

The Government of the United States 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 staffs, equipment purchasing and installation and initiation of research activities. The second interim report covered from January to June, 1986. Main emphasis focused on research activities and germplasm collection and administrative work.

II. RESEARCH ACTIVITIES

During June, 1986 to December, 1986. Research has been continuing effort to develop tissue culture system for mass propagation *in vitro* conservation and germplasm collection. Progress could be summarized as follow:-

1. DEVELOPMENT OF TISSUE CULTURE TECHNIQUE FOR MASS PROPAGATION.

1.1 Stem-bud segments system:

From earlier report, research was concentrated on the establishment of cultures. Success, to a certain extent, has been reported. Research was focused on initiation and multiplication of cultures. It could be summarized as follow:

(1). Further research on establishment of cultures (initiation).

Table 1 summarizes result of futher research work on bud break of stem-segment cultures. Responses of stem-segments were depended upon species and medium formular.

Table 1. Responses of Bamboo Stem-segments cultured to various media.

Species	Clones or local name	% Bud-break on various media*					Comments
		WP	MS	W_1	M_1	I	
Arundinaria pusilla	1	37.5	12.5	37.5	62.5	75	
" "	2	-	50	50	75	25	
A. suberecta	1	33.3	-	-	33.3	33.3	
" "	2	20	50	16.7	33.3	-	
Bambusa burmanica	1	100	100	50	100	66.7	
" "	2	-	-	-	-	-	Dormant buds.
B. burmanica	Van Bai Lek	-	14	-	-	-	
B. glaucescens	1	66.7	50	27.3	75	-	
" "	2	66.7	100	57.1	-	-	
" "	3	100	-	18.23	100	25	
" "	4	35	35	40	60	40	
B. glaucescens f.solida	-	56.3	85.7	7.1	71.4	75	
B. longispatha	-	-	-	-	-	100	
B. polymorpha	-	-	-	100	25	-	
B. ventricosa	-	-	100	33.3	-	33.3	
B. ventricosa variegatus	-	40	-	40.91	14.28	-	
B. vulgaris variegatus	-	100	-	-	100	100	
Cephalostachyum pergracile	1	-	25	-	-	-	Death
" "	2	-	-	66.7	-	-	Cultured on W_1 only
C. virgatum	-	-	-	50	-	-	
Dendrocalamus sp.	Sang-Konkaen	-	8.3	-	25	-	
" "	Sang-Nongkai	-	60	-	-	60	
" "	Sang-Esan	-	50	75	-	75	
D. asper	Tong-Moa 1	-	100	42.8	57.1	14.2	
"	Tong-Dam	20	25	-	20	-	
"	Tong-Keaw	44	44	-	-	44	
"	Tong-Moa 2	-	-	-	-	-	Death

Table 1. (Cont'd.)

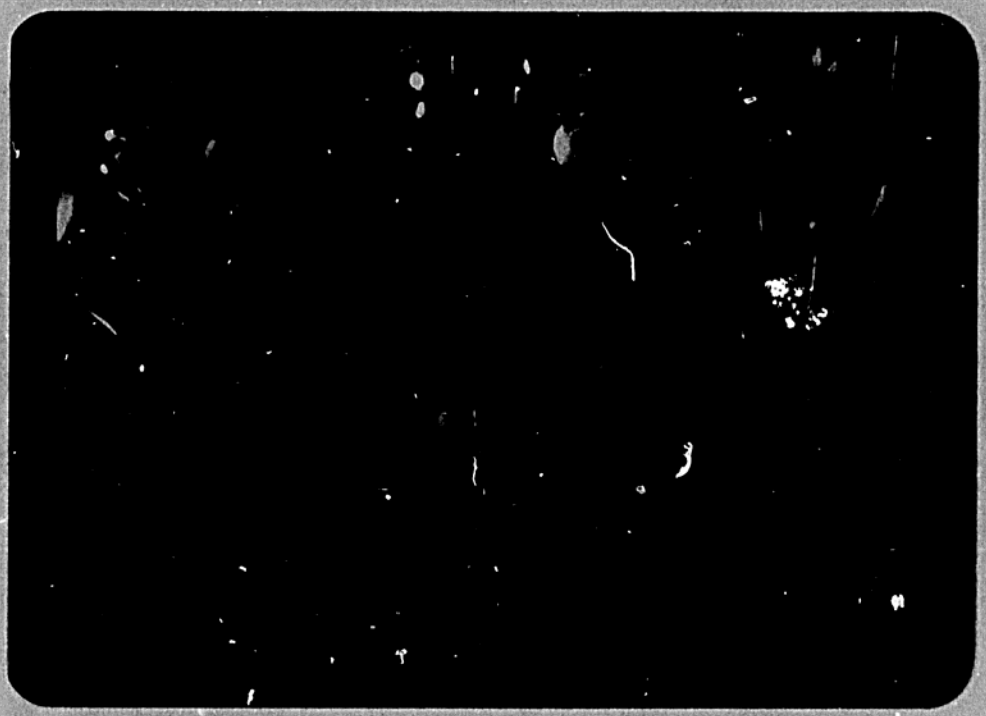
Species	Clones or local name	% Bud-break on various media*					Comments
		WP	MS	W_1	M_1	I	
D. gianteus	-	-	-	-	-	-	Death
D. membranaceus	-	40	40	20	20	-	
D. strictus	-	-	9.1	-	-	25	
Gigantochloa aspus	Ta-Guang	-	-	-	-	-	Death
Hibanobambusa tranguillans	-	83.3	50	25	50	-	
Phyllostachys aurea	-	-	-	-	-	-	Dormant buds.
P. bambusoides	-	27.2	14.3	12.5	-	-	
P. humilis	-	50	33.3	37.5	60	-	
P. nigra	-	-	-	-	-	-	Dormant buds.
P. nigra f.megurochiku	-	90	50	55.5	55.5	62.5	
P. gramineus	-	27.2	50	60	28.6	57.1	
Pleioblastus fortunee	-	-	-	37.5	-	-	
Pseudosasa japonica f. tsutumiana	-	60	14.3	20	37.5	28.5	
Sasaella suwekoana	-	-	-	100	100	-	
Semiarundinaria fastuosa	-	62.5	57.1	-	11.1	40	
Thyrsostachys siamensis	-	40	-	100	100	-	
T. siamensis	Rauk-Dang	33.3	-	33.3	50	-	
T. oliveri	-	-	-	100	25	-	

* WP = Woody plant medium (1981).
MS = Murashige and Skoog (1962).
W_1 = WP + 2 mg/l BA + 1 mg/l NAA + 2% sucrose.
M_1 = MS + 2 mg/l BA + 1 mg/l NAA + 3% sucrose.
I = MS + 5 mg/l BA + 1 mg/l NAA + 4% sucrose + 0.3% activated charcoal.

(2). Research on multiplication. It could be summarized as follow: -

a. When a single shoot of a clump consisted of several shoots was separated and cultured, most of them died, that might be due to damage during shoot separation.

b. When a large clump was separated into a small clump consisted of 3-4 shoots and a part of stem portion, multiple shoots formed and grew to a larger clump which could be further divided (Picture 1). However, the responses varied from medium to medium.



Picture 1. Multiple shoots.

(A) *Bambusa glaucescens.*

(B) *Bambusa nana.*

c. The most suitable medium for multiple shoots formation was a Murashige and Skoog (1962) medium supplemented with 2 mg/l 6-Benzylamino purine and 1 mg/l α- Naphthaleneacetic acid.

d. 3% or 4% sugar was suitable for bud break.

e. Multiple shoots occured by direct axillary bud enhancement from the existing shoot (Picture 2 A). Another phenomenon, the multiple shoots occured at the basal portion of a clump (Picture 2 B).

Picture 2. Multiple shoots formation.

(A) Direct axillary bud enhancement.

(B) Multiple shoots formed at the basal portion of the clump.

f. When shoot was separated from the clump and cultured onto a WP medium supplemented with 3 mg/l IBA and 2 mg/l NAA, root formed (Picture 3).

Picture 3. The different characteristic of shoots of *Bambusa glaucescens*.

(A) Multiple shoots form.

(B) Rooted shoots form.

g. When a stem-node segment of *in vitro* bamboo was excised and re-cultured onto the MS medium supplemented with 2 mg/l BA and 1 mg/l NAA, bud broke and grew. Thus, it could be multiplied 9 times within 3 months.

(3). Rooting of shoots. Further research were conducted to induce rooting of bamboo shoots. After a few experiments, research results could be concluded as following: -

a. *In vitro* stem-bud segments excised from field stock plant produced sometimes root in the MS medium containing 2 mg/l BA and 1 mg/l NAA or 3 mg/l IBA and 2 mg/l NAA.

b. Similar response was observed on shoots arose from stem-bud segments of aseptic cultures. However, the response was more readily than the bud excised from the field (Picture 4).

c. Time requirement for shoot to produce root varied from species to species, from clone to clone and depended on medium formular. Table 2 shows time requirement for rooting of shoots.

Picture 4. Root formation of *Thyrsostachys siamensis*.

(A) shoots from in vivo stem-bud segment.

(B) shoots from in vitro stem-bud segment.

Table 2 Time requirement for shoot to produce root (days).

Species	No. of Days requirements	Medium*	Comment
Arundinaria suberecta	117	I	from *in vivo* stem-bud segments.
Bambusa flexuosa	59	M_1	"
B. glaucescens f.solida	39	I	"
" "	42	MS	"
B. ventricosa	149	I	"
B. vulgaris (clone 1)	146	I	"
B. vulgaris variegatus	43	I	"
Pseudosasa japonica f.tsutumiana	52	M_1	"
Thyrsostachys siamensis (clone 1)	125	M_4	"
T. siamensis (clone 3)	128	M_4	"
B. ventricosa	84	I	from *in vitro* stem-bud segments.
B. vulgaris	102	WP,M_1	"
B. vulgaris	85	I	"
T. siamensis (clone 3)	62	M_1	"
" "	111	W_1	"

* MS = Murashige and Skoog (1962).

M_1 = MS + 2 mg/l BA + 1 mg/l NAA + 3% sucrose.

M_4 = MS + 2 mg/l BA + 1 mg/l NAA + 4% sucrose.

WP = Woody plant medium (1981).

W_1 = WP + 2 mg/l BA + 1 mg/l NAA + 2% sucrose.

I = MS + 5 mg/l BA + 1 mg/l NAA + 4% sucrose + 0.3% activated charcoal.

d. When shoots of *Bambusa sp.* were cultured onto a MS medium containing 3 mg/l IBA and 2 mg/l NAA. Root started to form within 23 days and progressively developed until 108 days. A high percentage of rooting (64.70%) was recorded by 108 days (Table 3).

Table 3. Root formation in *Bambusa sp.* (Chiang Mai).

Days after sub-culture	Percent of explants
23	5.88
28	17.65
42	23.53
52	29.41
57	47.05
70	52.94
82	58.82
108	64.70

1.2 Leaf callus system:

During this report period, research was concentrated on callus induction, callus growth and regeneration into plantlets. Medium formular as well as growth regulators effects were the main parameters in the studies. The following is the summary of the findings: -

a. In addition to the previous report (table 5 page 16), the follow species were included in the experiment during this report period.

Table 4. Bamboo Species.

Source	Species	Callus formation	
		Underground shoot	Aerial shoot
Bangkok	*Arundinaria glaucescens*	/	-
	Bambusa sp. (Pai-Van*)	-	/
	Bambusa vulgaris Schrad.	/	-
	Dendrocalamus asper Backer. Syn. *Gigantochloa asper* Kurz. *Bambusa aspera* Schult.	/	-
	Dendrocalamus asper Backer. (Pai Tong-Keaw*)	/	-
	Phyllostachys sulphurea	/	-
Chiangmai	*Bambusa flexuosa* Munro.	/	-
	Bambusa tulda	/	-
	Cephalostachyum pergracile Munro.	-	/
	Dendrocalamus asper Backer. (Pai Tong-Van*)	/	-
	Thyrsostachys siamensis (Pai Rauk-Dang*)	-	/
	Gigantochloa albociliata Kurz.	-	/
Kanchanaburi	*Dendrocalamus strictus*	/	-
	Thyrsostachys siamensis Gamble.	/	-
Prajinburi	*Dendrocalamus asper* Backer (Pai Tong-Van*)	/	-
Pathumthanee	*Bambusa flexuosa* Munro.	/	/
Nakornracha-sima	*Bambusa sp.* (Pai Van-Ton*)	/	-
	Bambusa sp. (Pai Van-Prong*)	/	-
	Bambusa pubescens Lind.	/	-

* Local name

b. Young leaf sheath was the most suitable for culturing to induce callus formation. Callus occured at the cut-edge and on the leaf surface (Picture 5).

Picture 5. Callus formation on the leaf sheath of *Phyllostachys sulphurea*.

1 = no callus

2 = slight callus formation

3 = average callus formation

4 = good callus formation

5 = exceptional good callus formation

c. Callus started to form as fast as within one week. In most case, if successful, callus formation occure within 1 month.

d. 2,4-D was proven to be a suitable callus induced agent. 2,4-D at 3 mg/l incorporated into the medium induced callus formation in several bamboo species.

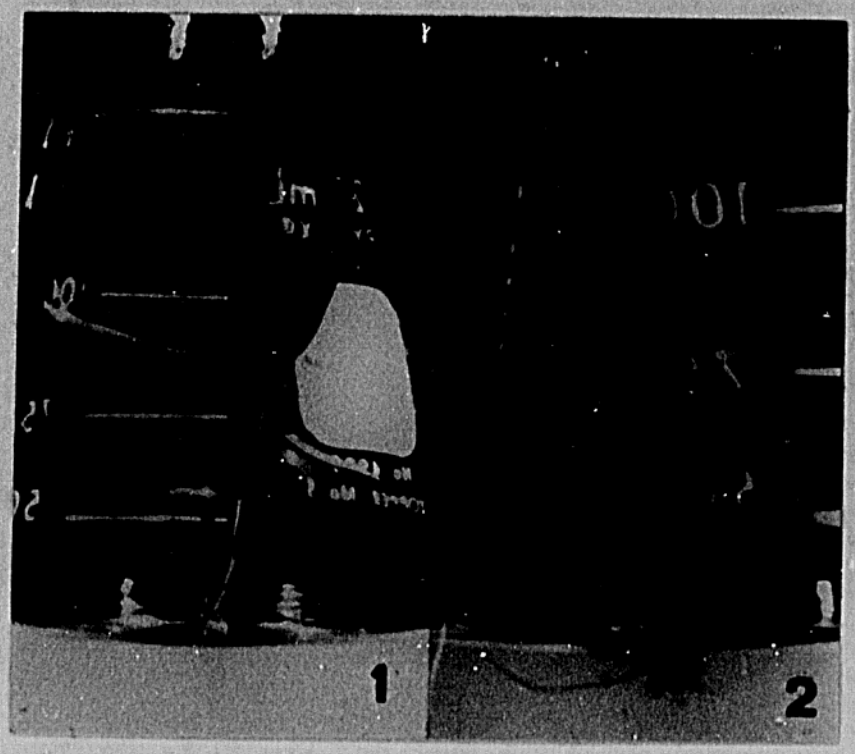
e. Callus could be subcultured and multiplied on the same medium (Picture 6).

Picture 6. Callus of *Phyllostachys sulphurea*.

f. In *Bambusa flexuosa* Munro., callus turned into embryogenic callus and continue to grew. In appropriate medium, embryogenic callus grew into plantlets (Picture 7).

Picture 7. Sequential development from callus (1) to embryogenic callus (2), small plantlets (3) plantlets (4&5). Arrow shows root formation.

g. When shoots were excised and cultured onto a rooting medium, root formed readily (Picture 8).



Picture 8. Root formation in *B. flexuosa*.

(1) shoots

(2) shoots with roots

1.3 Seed system:

Research was continued from the previous report. It was focused on multiple shoots formation in order to multiply in large quantities. The following bamboo species were included:

Bambusa arundinacea (Retz) Willd.

Dendrocalamus strictus Nees.

Dendrocalamus membranaceus Munro.

a. Cytokinins effect was investigated. It was found that BA was superior to kinetin and 2ip, except in *Dendrocalamus strictus*. Kinetin effect was comparable to BA.

b. An average of 4 shoots, 6.5 shoots and 11 shoots could be induced from one seed at one time in *Bambusa arundinacea*, *Dendrocalamus strictus*, *Dendrocalamus membranaceus* respectively.

c. Addition of NAA into the medium had no effect on shoot formation.

2. DEVELOPMENT OF TISSUE CULTURE TECHNIQUE FOR IN VITRO CONSERVATION.

Research to develop a tissue culture technique for in vitro conservation was, in parallel to sub-culture technique which was developed simultaneously with tissue culture for mass propagation, a minimum growth technique was concentrated. The followings were the summary of research:-

2.1 Stem-bud segments system.

Low temperature.

Experiment has been conducted to evaluate the effect of low temperature and length of incubation of *B. glaucescens*'s stem-bud segments. Experiment is in progress. Preliminary data suggested that tissue of *B. glaucescens* could be kept as low as $5^{o}c$. Height was reduced 24 times from the control. After 3 months, most of the plants survive at $5^{o}c$ and $10^{o}c$ but less survival at $25^{o}c$.

2.2 Leaf callus system.

During this report period, callus was inadequate amount for conservation experiment. Callus is multiplying. As soon as, adequate amount of callus is obtained, similar experiment to stem-bud segments is planned to conducted.

2.3 Seed system.

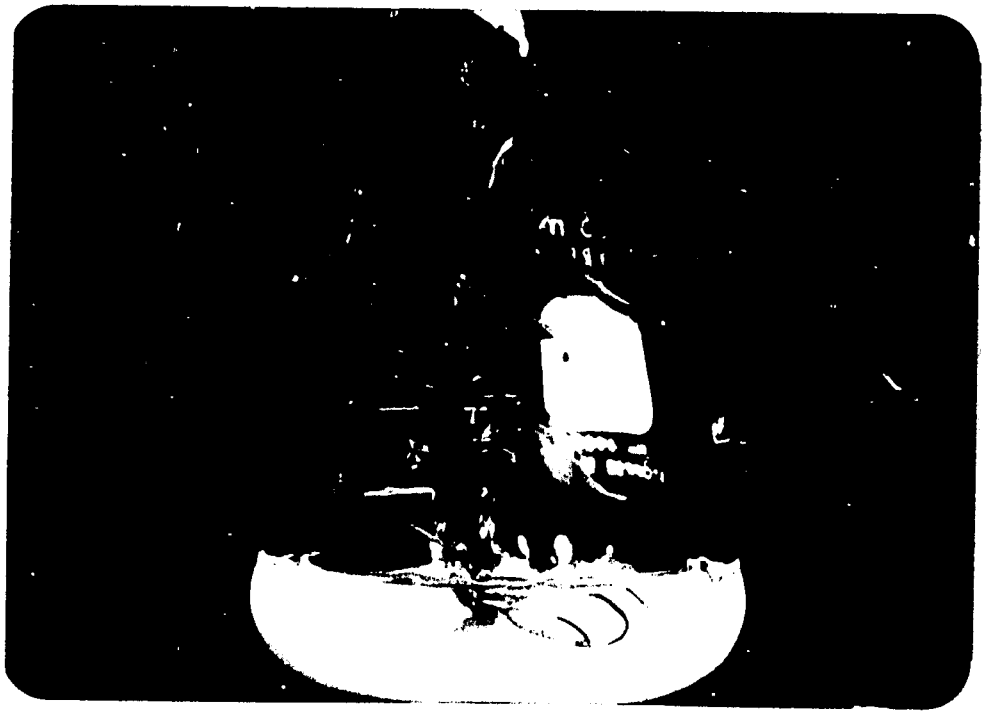
Effect of growth retardant, CCC and Daminozide was further investigated. Seedlings, 7 days old, *Dendrocalamus membranaceus* Munro.were used. The following findings were summarized.

a. Table 5 shows the effect of Daminozide at different concentrations. Height of seedlings could be reduced as much as 50% of the control. Survival percentage was low in Daminozide treatment (52.63), while all control seedlings survived after 3 months.

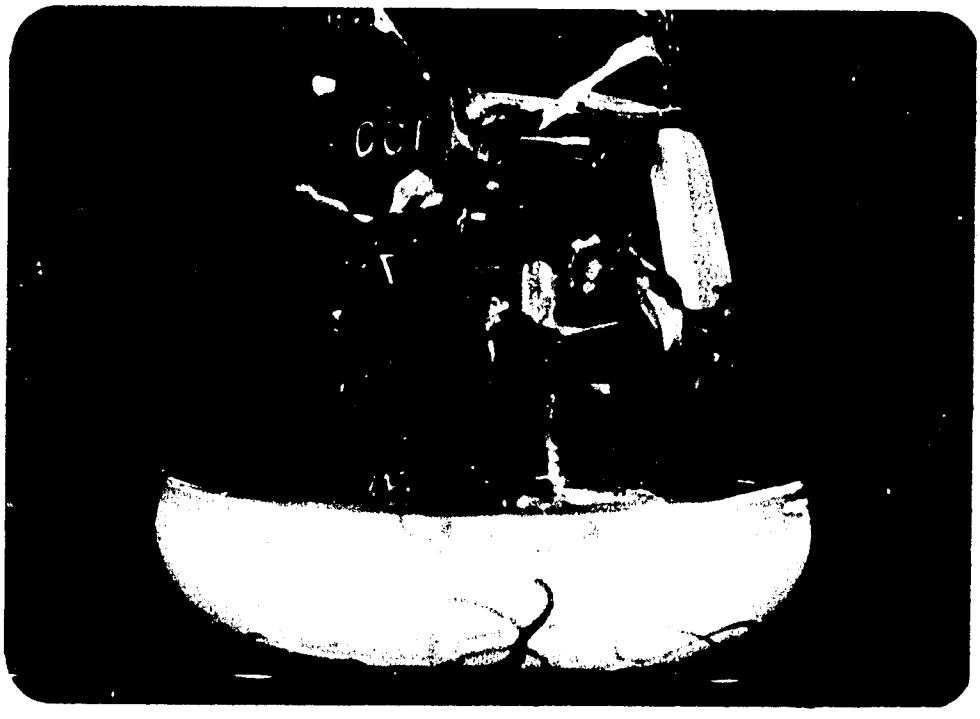
Table 5. Effect of Daminozide on Height and Survival of seedlings of *Dendrocalamus strictus* Nees.

Treatment	Height		% survival after 3 months
	average (cm)	%	
Daminozide 0 mg/l	9.37	100	100
Daminozide 500 mg/l	6.94	74.07	100
Daminozide 1,000 mg/l	6.66	71.08	83.78
Daminozide 2,000 mg/l	5.65	60.30	54.90
Daminozide 3,000 mg/l	4.86	51.87	52.63

b. It was surprised that at high concentration of Daminozide, seedlings tended to produce more shoots (Picture 9, 10). Futher investigation is inprogress.



Picture 9. Multiple shoots of *D. strictus* Nees. formation at 1,000 mg/l Daminozide treatment.



Picture 10. Multiple shoots of *D. strictus* Nees. formation at 2,000 mg/l Daminozide treatment.

III. GERMPLASM COLLECTION

Up to date, eighty-one clone of forty-one species, represent 13 genus, were collected in Thailand. All of them were cultured onto a define medium. Thirty-two clones, represent twenty-two species and 7 genus have survived in cultures. The rest has not been successful in cultures. Attempt to maintain those bamboo in cultures and to culture again is in progress. Table 6. shows bamboos which were collected and cultured.

Table 6. Status of Bamboo Collection and Cultures. (from June to October, 1986.)

Date of collection	Species	Clones	Places of collection	Status incultures		Comments 1/
				survive	death	
June 4, 1986	*Bambusa burmanica*	Van-Lek	Saraburi	/		Do
	Cephalostachyum pergracile	-		/		Do
	Dendrocalamus strictus	Sang-Konkaen			/	
	D. strictus	Sang-Nongkai		/		
June 6, 1986	*D. asper Backer*	Tong-Van	Prajinburi	/		C
June 23, 1986	*Arundinaria pusilla*	-	Bangkok	/		
	A. suberecta	-		/		
	Bambusa ventricosa	-		/		
	Dendrocalamus giganteus	-		/		
	D. membranaceus	-			/	
June 25, 1986	*Dendrocalamus asper*	Tong-Moa	Kanchanaburi		/	C
	D. strictus	-		/		Do
	Thyrsostachys siamensis	-		/		
July 2, 1986	*T. siamensis*	-		/		
July 7, 1986	*Arundinaria suberecta*	-	Bangkok	/		
	Bambusa glaucescens	-		/		
	B. ventricosa	-		/		

Table 6. (Cont'd.)

Date of collection	Species	Clones	Places of collection	Status incultures		Comments 1/
				survive	death	
August 8, 1986.	*Phyllostachys humilis*	-		/		
	P. gramineus	-			/	
	Phyllostachys nigra f. megurochiku	-			/	
	Pseudosasa japonica f. tsutumiana	-			/	
	Sasaella suwekoana	-			/	
	Semiarundinaria fastuosa	-		/		
August 13, 1986.	*Bambusa glaucescens*	-	Nontaburi	/		
September 9, 1986.	*Arundinaria pusilla*	-	Bangkok	/		Do
	Dendrocalamus asper	Tong-Dam			/	
	D. asper	Tong-Keaw		/		Do
September 15, 1986.	*D. asper* Backer.			/		C
September 16, 1986.	*Bambusa sp.*	Pai-Keaw		/		
September 22, 1986.	*B. arundinacea*	-	Kanchanaburi	/		
	Dendrocalamus strictus	-		/		P
	Thyrsostachys siamensis	-		/		
October 1, 1986.	*Bambusa flexuosa* Munro.	-	Chiangmai	/		C
	B. tulda	-		/		C
	Cephalostachyum pergracile Munro.	-		/		C

Table 6. (Cont'd.)

Date of collection	Species	Clones	Places of collection	Status incultures		Comments 1/
				survive	death	
July 17, 1986	*Arundinaria pusilla*	-	Bangkok	/		
	Dendrocalamus asper	Tong-Dam		/		Do
	D. asper	Tong-Keaw		/		
July 22, 1986	*Bambusa ventricosa variegatus*		Chiangmai	/		
	B. polymorpha	-				
	Cephalostachyum pergracile	-			/	
	C. virgatum	-			/	
	Gigantochloa apus Kurz.	-			/	
	Thyrsostachys siamensis	-		/		
July 25, 1986	*Dendrocalamus strictus*	-	Kanchanaburi	/		C
August 1,1986	*Phyllostachys sulphurea*	-	Bangkok	/		C
August 8,1986	*Arundinaria suberecta*	-			/	
	Bambusa glaucescens f. solida	-		/		
	B. vulgaris	-		/		
	Hibanobambusa tranguillans	-			/	
	Phyllostachys aurea	-			/	
	Pleioblastus fortunee	-			/	
	Phyllostachys bambusoides	-		/		
	P. nigra	-		/		

Table 6. (Cont'd.)

Date of collection	Species	Clones	Places of collection	Status incultures		Comments 1/
				survive	death	
October 1, 1986.	*Dendrocalamus asper*	-	Chiangmai	/		C
	Gigantochloa albociliata	-		/		C
October 10, 1986.	*Bambusa sp.*	Pai-Van	Bangkok	/		C
October 30, 1986.	*Bambusa sp.*	Pai Van-Ton	Nakornrachasima	/		C
	B. sp.	Pai Van-Prong		/		C
	B. pubescens Lind.	-		/		C

1/ = Comments.

C = Callus formation in vitro.

Do = Dormant (buds or culms) in vitro or in vivo.

P = Partially died in vitro.

IV. FUTURE WORK PLAN FROM JANUARY TO JUNE, 1987.

1. TECHNOLOGY DEVELOPMENT.

1.1 Stem-bud segments system.

- continue experiment on multiplication and rooting.
- experiment on transplanting.
- continue study the effect of low temperature and growth retardants on conservation.
- initiate cryo-preservation experiments.

1.2 Leaf callus system.

- continue experiment on multiplication and regeneration.
- experiment on transplanting.
- if callus is adequate, experiment on conservation similar fashion to stem-bud segments system will be conducted.

1.3 Seed system.

- apply shoot multiplication system of other species.
- continue experiment on the effect of low temperature, growth retardant for conservation of other species.

2. GERMPLASM COLLECTION.

- continue to collect bamboo species in Thailand, especially those did not survive in earlier collection.
- start to collect bamboo germplasm in Sri Lanka after collaboration arrangement is done in January, 1987.

V. CONCLUSION

Research activities have been progressively well. Tissue culture development for mass propagation is satisfactory successful, though not all species could be cultured. It shows viable technique for bamboo propagation. Several experiments are still needed to refine the compositions of the medium, technique and to complete tissue culture protocol.

Research to develop *in vitro* conservation technique have progressed well and moved to the right direction, Several experiments are indeed needed during the next period prior to draw-up a conclusion

Germplasm collection in Thailand are going very well. Almost all species have been collected, though not all of them survived. Locations have been identified, thus, it is now easy to collect them.