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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/9/88

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. 5, 2/7
2d Interim Report

Attachment

2nd INTERIM REPORT

FOR

IN VITRO CONSERVATION AND GERMPLASM EXCHANGE OF BAMBOO

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JUNE 30, 1986.

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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. This interim report covers from January to June, 1986. Main emphasis has been focused on research activities and germplasm collection and administrative work.

II. RESEARCH ACTIVITIES

During period January to June, 1986. Research has been intensively conducted to develop the technologies (systems or protocols) for mass propagation and *in vitro* conservation. Three systems, as reported earlier in the first interim report, namely; stem-bud segments, leaf callus and seed, have been investigated in order to develop the above said technologies. Three major unforseen problems arose during this period, they are:-

1. From January to April, 1986, was the dry season which bamboo became dormant. Branches or shoots were limited for experimental materials.
2. It was found that dormant buds tended to continue theirs dormant in cultures
3. It was also observed that heavy contamination was associated with the dormant tissues.

To cope with the problems above, plant materials from various sources in Thailand were collected from time to time. Those areas included Kanjanaburi province (200 km west of Bangkok), Chiangmai (North), Pakchong (North-East), Prachinburi (East), Chitlada Palace (Bangkok) and various places in Bangkok metropolitan.

Surface sterilization techniques has to be modified from one lot of plant materials to the others depending upon the location and types of bamboo. Special treatment to the explants prior to culture was sometimes needed to reduce the contamination problem.

EXPERIMENTAL RESULTS COULD BE CUMMERIZED AS FOLLOW:-

1. DEVELOPMENT OF TISSUE CULTURE TECHNIQUE FOR MASS PROPAGATION.

1.1 Stem-bud segments system:

From a series of experiments the following results were the conclusion of the findings; they are:

a. Medium: The most suitable medium for initiation was a woody plant medium (WP)* supplemented with 2 mg/l 6-Benzyladenine (BA) and 1 mg/l α- Napthaleneacetic acid (NAA) (Call "W_1"). However, it seemed unsuitable to prolong cultures since tissues turned brown. Experiments are in progress to search for the suitable medium for long term culture.

b. Explants: It was found that the explants excised from mature branch or shoot (just turn to become mature) were the most suitable tissue because the explants survived at a higher percentage, higher bud-break and higher number of shoots per bud, than explants taken from the young ones (Table 1).

Due to heavy contamination and buds tended to remain dormant, using knife cleaning around the buds and remove the leaf scale prior to surface sterili sterilization was necessary to reduce contamination and enhance bud-break.

* G. Lloyd and B.H. McCown. 1981. Commercially feasible micropropagation of Mountain Lanrel, Kalmia latifolia, Linn. by used shoot-tip culture. Proc. Intern. Plant Prop. Soc. 30: 421-427.

Table 1. Effects of mature and young materials of *Bambusa vulgaris* on survival rate, bud-break, number of shoots per bud and browning

Kinds of tissue	Time (wks)	Survival rate (%)	Bud-break (%)	Number of shoots per bud	Browning (%)
Mature tissue	1	88.2	58.82	1.8	90.6
	2	82.35	58.82	2.1	48.9
	3	82.35	64.71	2.8	90.5
	4	82.35	70.58	3.45	100
	5	58.82	76.47	3.5	100
Young tissue	1	62.9	-	-	90.7
	2	40.3	3.85	1	90.1
	3	33.9	3.85	1.6	100
	4	33.9	7.69	2.5	100
	5	6.45	11.54	2.67	100

*/ *Bambusa vulgaris*

3. Surface sterilization technique: As mentioned earlier that the technique has to be modified from time to time depending on plant materials. However, the following method was generally used in our laboratory; dipped plant materials in 70% Ethanol for 30 seconds after cleaning the explants, then soaked in 20% clorox solution for 20-30 minutes, rinsed two times with sterilled distilled water.

4. Contamination: Contamination occured from less than 10% up to 100% depended upon what, where and when plant materials were taken (Table 2). Sometimes, surface sterilization technique did not reduce contamination problem due to heavy infection.

5. Browning and survival rate: Most of the bamboo tissue released chemicals into the medium, as shown by brown color. Degree of browning (Picture 1) varied from species to species (Table 3), ages of explants (Table 1) and interaction with the medium (Table 4). The survival rate was highly associated with degree of browning. The higher the degree of browning, the higher the mortallity rate. Frequency of sub-culture up to everyday was, till now, not helpful.

6. Bud-break from the stem-bud segments: Bud-break percentages varied from one group of plant materials to the others and from species to species. With a suitable technique and suitable medium, up to date, bud-break could be induced upto 75%. The number of shoots per buds varied from 1 to 4 shoots (Table 1). Shoot growth and development have been divided into 4 stages (Picture 2), which will be used as a criteria for future experiments.

Table 2. Contamination as effected by plants materials and locations.

Locations of collection	Kinds of bamboo	Contamination(%)
Kanjanaburi	*Bambusa burmanica*	16.67
	Dendrocalamus longispathus	50
	Oxytonanthera albociliata	76.92
Chiangmai	*Arundinaria subcrecta*	0
	Bambusa longispatha	60
	B. ventricosa (clone 1)	7.1
	B. ventricosa (clone 2)	60
	B. arundinacea	52.38
	B. polymorpha Munro	12
	Thyrsostachys siamensis(clone 1)	13.89
	T. siamensis (clone 2)	0
	T. siamensis (clone 3)	0
	T. siamensis (clone 4)	30.43

Table 2. (cont'd.)

Locations of collection	Kinds of bamboo	Contamination (%)
Prachinburi	*B. glaucescens* (clone 1)	10.53
	B. glaucescens (clone 2)	11.76
	B. longispatha	26.67
	B. burmanica	38.89
	B. burmanica (Leui)*/	33.33
	B. nana	16.67
	D. membranaceus	66.67
	D. asper (Tong Moa)*/	47.62
	T. siamensis (Rauk Daeng)*/	13.33
Bangkok	*B. glaucescens* (clone 1)	26
	B. glaucescens (clone 2)	8.33
	B. vulgaris (clone 1)	32.25
	B. vulgaris (clone 2)	41.17
	B. vulgaris (Thong Yai)*/	46.15
	D. giganteus	81.25
	P. sulphurea (clone 1)	100
	P. sulphurea (clone 2)	54.2

*/ Local name.

Table 3. Percents of browning from a bamboo tissue within 2 months.*/

Kinds of bamboo	Browning (%)
Arundinaria sulerecta	100
Bambusa longispatha	60
B. ventricosa (clone 1)	100
B. ventricosa (clone 2)	100
B. arundinacea (clone 1)	66.67
B. arundinacea (clone 2)	90
B. glaucescens	92.86
B. nana	93.67
B. vulgaris	100
B. vulgaris (Thong Yai) **/	100
B. polymorpha Munro	91.67
Phyllostachys sulphurea	100
Thyrsostachys siamensis (clone 1)	61.1
T. siamensis (clone 2)	100
T. siamensis (clone 3)	100
T. siamensis (clone 4)	71.74

*/ in W_1 medium.

**/ Local name.

Table 4. Browning of a bamboo tissue in various kinds of medium within 1 month.

Kinds of bamboo	Browning in various medium (%) */				
	MS	M_1	WP	W_1	I
Bambusa glaucescens (clone 1)	-	33.3	-	33.3	0
B. glaucescens (clone 2)	-	75	100	33.3	0
B. longispatha	100	50	50	33.3	0
B. burmanica	-	-	-	-	0
B. burmanica (..eui)	50	100	100	100	0
B. nana	-	25	-	-	0
Dendrocalamus membranaceus	-	-	-	-	0
D. giantcus	-	-	-	-	0
D. asper (Tong Moa) **/	-	66.7	-	-	0
Thyrsostachys siamensis (Rauk Daeng) **/	-	-	100	-	0

*/ MS = Murashige and Skoog (1962).

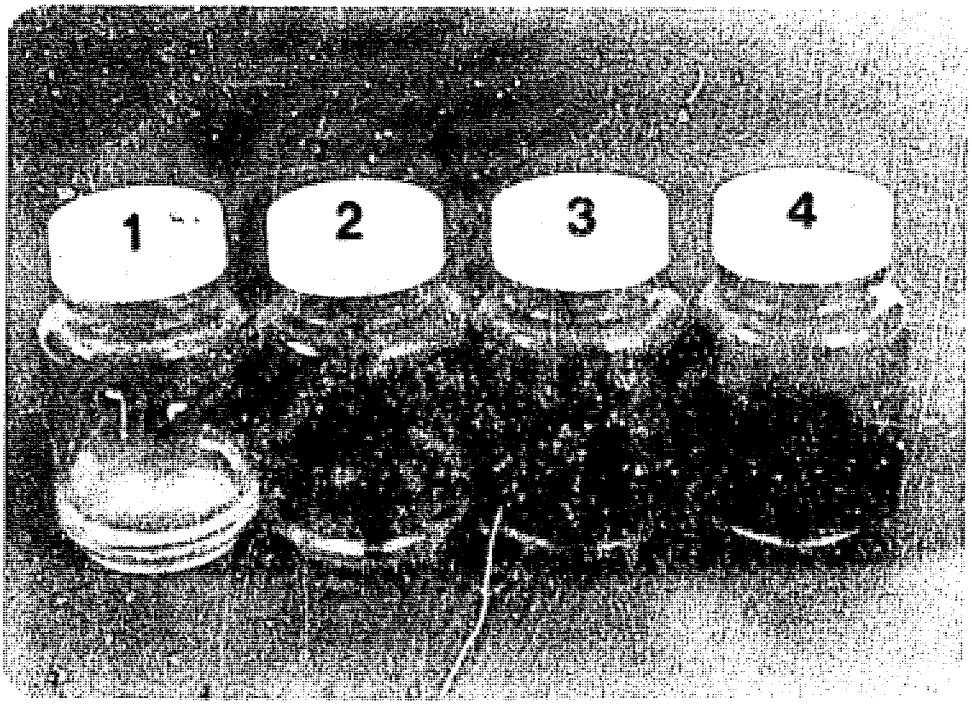
M_1 = MS + 2 mg/l BA + 1 mg/l NAA.

WP = Woody plant medium.

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

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

**/ = Local name.



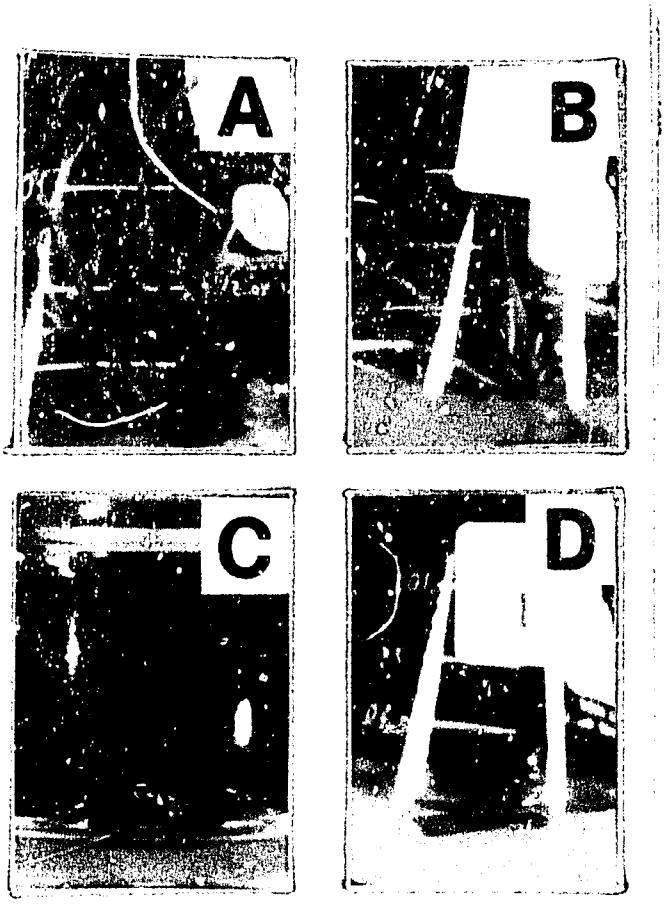
Picture 1. Degree of medium browning after culturing bamboo tissue.

1 = no browning

2 = slight browning

3 = moderate browning

4 = severe browning



Picture 2.

A = shoot developed over 2 cm in height, leaf expanded.

B = shoot developed over 2 cm in height but leaf unexpanded.

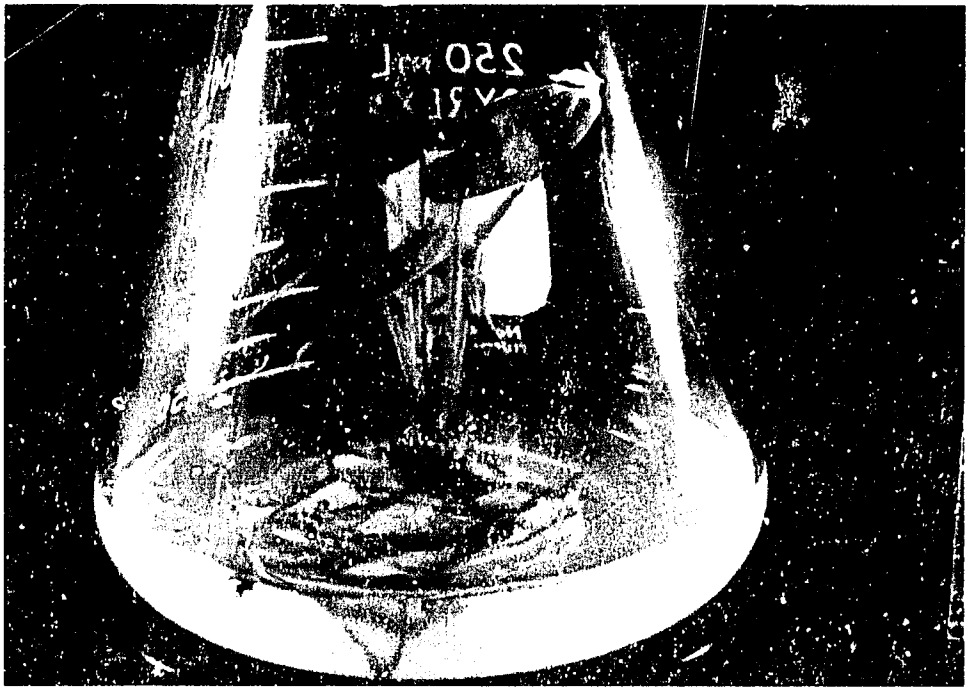
C = shoot developed less than 2 cm in height leaf unexpanded.

D = shoot just developed.

7. Multiple shoots formation: When a stem-bud segments was cultured on W_1 medium, bud was induced to break. Multiple shoots, in many cases, arose simultaneously. To maintain and multiply these shoots, experiments are in progress. Preliminary result suggested that shoots have to be cut-off from the stem segments and sub-cultured onto MS medium supplemented with 1 mg/l NAA, 2 mg/l BA and 4% sucrose (instead of 3%), then subcultured back to W_1 medium after 1 month. Multiple shoots could be promoted (Picture 3).

8. Rooting of shoots: Due to limited number of shoots for rooting experiments, two trials have been tested. Thirty-seven percents of shoots produced roots (Picture 4) within 1 month after they were cultured onto woody plant medium supplemented with 3 mg/l Indolebutyric acid (IBA) and 2 mg/l NAA.

Picture 3. Multiple shoots formation from a stem-bud segment culture of *Bambusa glaucescens*.



Picture 4. Root formation from a stem-bud segment culture of *Bambusa glaucescens*.

1.2 Leaf callus system.

From series of experiments, findings may be summarized as follow:-

a. Explants: Young leaf tissues from shoots arose from the soil (underground shoots) and shoots arose from stem (aerial shoots) were suitable for culturing. Callus was obtained about 30 days after innoculation (Table 5, Picture 5).

b. Medium: The most suitable medium to date is a woody plant supplemented with 2,4 -dichlorophenoxyacetic acid (3-80 mg/l), thiamine. HCl (10 mg/l), 0.3% activated charcoal and 3% sucrose.

c. Sterilization technique: Contamination problem in leaf cultures was less than stem-bud segments cultures. Therefore, normal procedure of surface sterilization was applied.

d. Browning and survival rate: Without exception, browning was still a major problem in leaf tissue cultures. Browning was highly associated with the survival rate. Sub-culturing and manipulating the medium to cope up with th se problems are in progress.

e. Callus multiplication: Experiment to investigate the growth of callus is in progress.

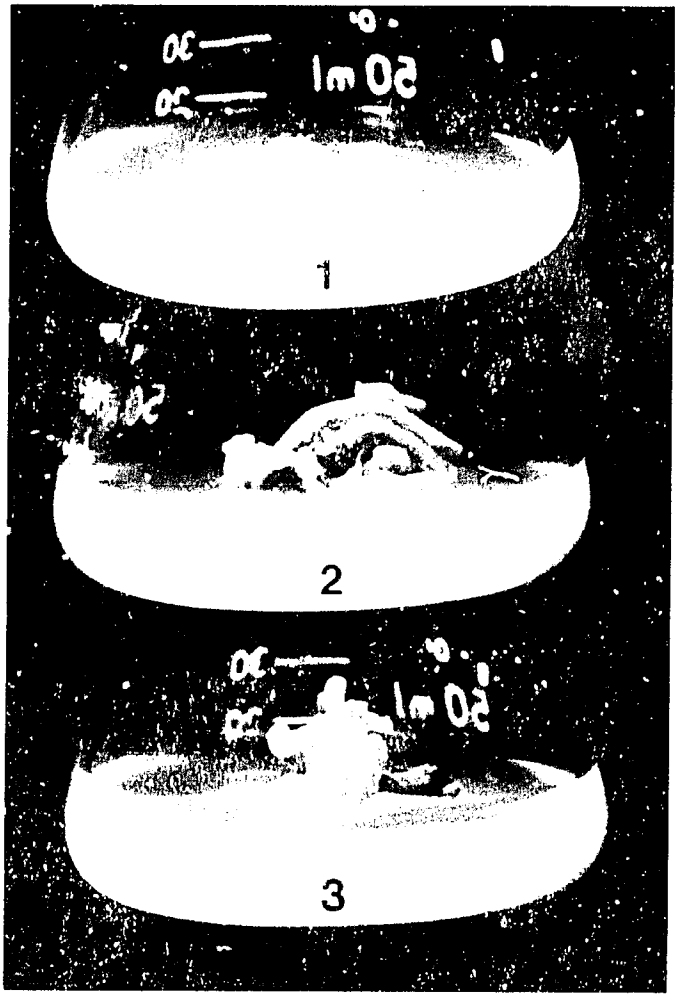
f. Plantlets formation: Attempt is being made to induce plantlets from callus by culturing callus in various combinations of auxin and cytokinins, various medium components, as well as in high auxins levels (up to 100 mg/l 2,4-D). Experiments are in progress. Preliminary result showed embryogenesis occuring on the callus of some species.

Table 5. Callus formation from underground and aerial shoots.

Kinds of bamboo	Callus formation	
	Underground shoot	Aerial shoot
Bambusa vulgaris Schrad.	/	.
B. nutans Wall	/	.
B. flexuosa Munro	.	/
B. ventricosa	.	/
Dendrocalamus hamiltonii	.	/
Phyllostachys sulphurea	/	.
Thyrsostachys siamensis (clone 1)	.	/
T. siamensis (clone 2)	/	/

= Callus formation.

= Unavailable materials.



Picture 5. Callus formation from young-leaf tissue of *Bambusa flexuosa* Munro. on a WP medium supplemented with 3 mg/l (1) 6 mg/l (2) and 9 mg/l (3) 2,4-D after 1 month.

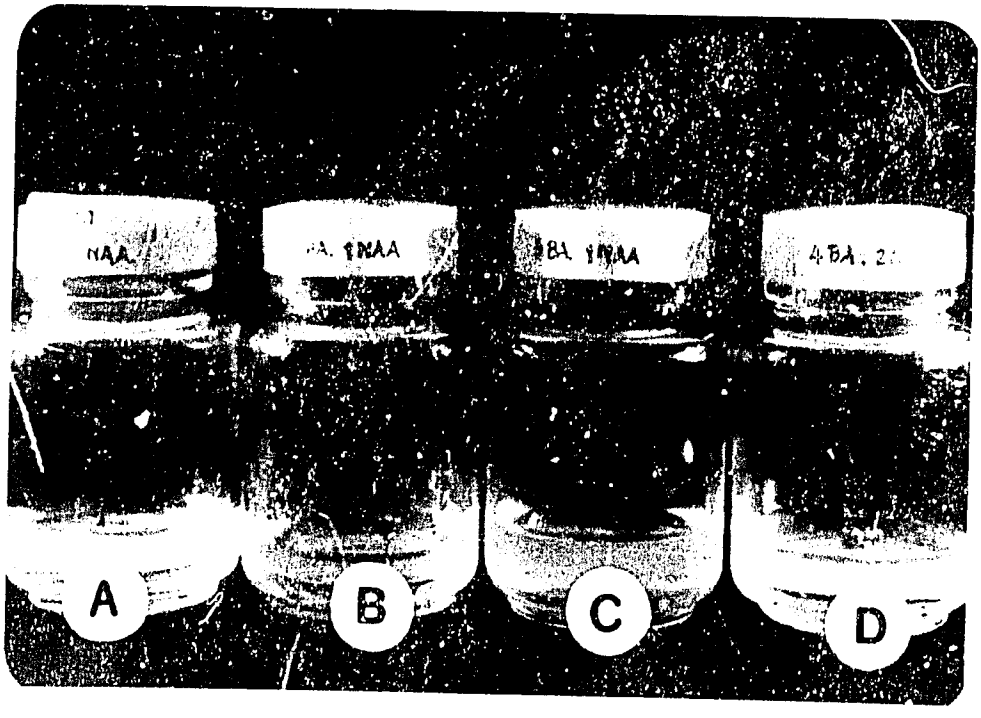
1.3 Seeds system.

Limited number of seeds were obtained from Department of Forestry, Kasetsart University. Attempts have been made to multiply plantlets from the seed germinated plants in vitro, which is another viable method for mass propagation. The following results were the summary of the highlight:

a. Seed germination. In general, seed germination was very low. By using in vitro propagation, high percentage of seed germination could be obtained. The most suitable medium amongst the tested media was a woody plant medium without growth regulators. Eighty-seven percents of seed germination could be acheived. However, single plantlet per seed was recorded. If seeds were germinated on medium containing growth regulators (auxins and cytokinins), multiple shoots per seed occured. Seeds which were germinated on a woody plant medium containing 4 mg/l BA and 2 mg/l NAA germinated at 82.5% and produced 2.8 shoots per seed on the average (Picture 6). Therefore, the seed germination medium was a woody plant medium supplemented with 4 mg/l BA and 2 mg/l NAA, instead of a woody plant medium alone.

b. Multiple shoots formation: Experiments were designed to further multiply shoot or shoots arose from the seeds (Picture 6). It was possible to induce multiple shoots from a single shoot by culturing it on a woody plant medium supplemented with BA and NAA. BA or NAA alone in the medium had lesser effect on multiple shoots formation than BA and NAA conbination. However, the result was not consistant. Kinetin and 2iP were not as effective as BA. In a similar manner; 2,4-D was not as effective as NAA, but it stimulated callus formation.

c. Root formation. It was found that shoots produced roots when they were cultured on 1-2 mg/l NAA enriched medium.



Picture 6. Seedling of *Bambusa arundinacea* (Retz.) Willd.

A. shoots formation on basal medium of Woody Plant (WP).

B. shoots formation on WP medium supplemented 2 mg/l BA and 1 mg/l NAA.

C. shoots formation on WP medium supplemented 3 mg/l BA and 1 mg/l NAA.

D. shoots formation on WP medium supplemented 4 mg/l BA and 2 mg/l NAA.

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

1.1 Stem-bud segments system:

In principle, basic techniques for explant establishment in cultures and multiplication of propagula, which have been highlighted in the aspect of development of tissue culture technique for mass propagation (see page 3-14), were similar works. At this moment, experiments for conservation aspects are being carried out to investigate the sub-culture frequency and growing periods. Minimum growth technique experiments by using low temperature and growth retardants are also conducting and in progress. Experiments on cryo-preservation by using shoots will be conducted in the near future.

1.2 Leaf callus system.

In the past 6 months, researches were conducted to produced callus, as mentioned earlier (see page 15-17). Callus of some bamboo species was multiplied. Callus is now being used as materials for minimum-growth experiments, and in the near future it will be used for cryo-preservation study. Experiments on minimum growth storage are in progress.

1.3 Seeds system.

Experiments to conserve bamboo seedlings have been conducted. These are the highlight of the findings:-

a. Seedlings could be maintained without sub-culturing for 3-4 months in normal conditions. It would be able to maintain for a long time by subculturing.

b. At 5 to 10°C, seedlings survived and grew slowly. It would be able to conserve about 3 months in the dark. If light was provided, it could be stored for more than 3 months.

c. At $0^{o}C$, seedlings died.

d. Seedlings growth at $5^{o}C$ and $10^{o}C$ was not significantly difference. Therefore, to conserve seedlings at $10^{o}C$ is prefered due to economical rearon.

e. When CCC was incorporated into the medium (Table 6, Picture 7), seedling growth was retarded depending upon CCC concentrations. The higher the concentration, the higher the degree of retardation. However, too high concentration was toxic to the seedling.

f. The most suitable concentration of CCC varied from bamboo species to species. CCC at 500-1,000 mg/l was suitable for *Thrsostachys siamensis* seedlings, while CCC at 1,000-2,000 mg/l was suitable for *Bambusa arundinacea* seedlings. Therefore, CCC at 1,000 mg/l is generally recommended.

g. Growth of seedlings was reduced about 50% when CCC at 1,000 mg/l was incorporated. Seedlings could be conserved for a long time. Experiment is in progress.

h. Similar to CCC, daminozide was used (Table 7, Picture 8) at 500 mg/l was the most suitable for *Thyrsostachys siamensis* and *Bambusa arundinacea* seedlings.

i. Daminozide at 500 mg/l reduced seedlings height about 50% when compared to the control. Seedlings could be conserved for a long period of time. Experiment is in progress.

Table 6. Effect of CCC on growth of *Bambusa arundinacea* (Retz.) Wild. and *Thyrsostachys siamensis* Gamble. after 8 months of culturing.

Woody plant medium containing	*Bambusa arundinacea*		*Thyrsostachys siamensis*	
	Height (cm)	Height comparison control = 100	Height (cm)	Height comparison control = 100
CCC 0 mg/l	14.0	100	10.0	100
CCC 250 mg/l	11.5	82	8.0	80
CCC 500 mg/l	10.0	71	7.0	70
CCC 1,000 mg/l	9.0	64	5.0	50
CCC 1,500 mg/l	8.0	57	D	0
CCC 2,000 mg/l	7.5	54	D	0
CCC 3,000 mg/l	5 (P)	36	D	0

(P) = Partially died

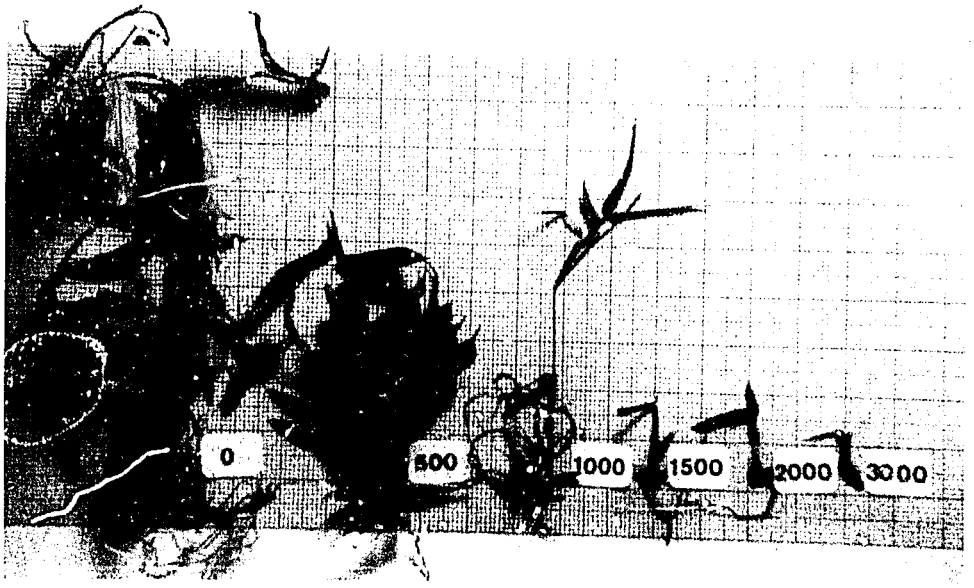
(D) = All died

Picture 7. Growth of *Bambusa arundinacea* on a woody plant medium containing 0, 250, 500, 1,000, 2,000 and 3,000 mg/l CCC after 8 months of culturing.

Table 7. Effect of daminozide on growth of *Bambusa arundinacea* (Retz.) Wild. and *Thyrsostachys siamensis* Gamble, after 8 months of culturing.

Woody plant medium containing	*Bambusa arundinacea*		*Thyrsostachys siamensis*	
	Height (cm)	Height comparison control = 100	Height (cm)	Height comparison control = 100
daminozide 0 mg/l	14	100	10	100
daminozide 500 mg/l	7	50	4	40
daminozide 1,000 mg/l	7	50	D	0
daminozide 1,500 mg/l	D	0	D	0
daminozide 2,000 mg/l	D	0	D	0
daminozide 3,000 mg/l	D	0	D	0

D = All died



Picture 8. Growth of *Bumbusa arundinacea* on a woody plant medium containing 0, 500, 1,000, 1,500, 2,000 and 3,000 mg/l Daminozide after 8 months of culturing.

III. BAMBOO GERMPLASM COLLECTION IN THAILAND.

Thai bamboo germplasm has been collected 29 times during the report period, covered Northern part, North East (partially), East and central plain. All materials were collected in 3 forms, whichever appropriate or both, they are;

1. culms
2. branches or shoots
3. seeds

Branches or shoots were collected for *in vitro* initiation. They can be used as a *in vitro* conservation materials in the future.

Culms were collected and planted at Horticulture Department, Kasetsart University, which will be used as a plant material sources.

Table 8 showed the species (sometimes clones) which have been collected from various places in Thailand, forms of collection and availability status.

Germplasm collection will be continued to gather all the existing species in Thailand and to cover as many parts of Thailand as possible.

Germplasm collection in Sri Lanka and collaboration has not been able to accomplished due to the political problem in Sri Lanka.

Table 8. Germplasm collection sheet.

Date of collection	Location	Species or Clones	Forms of collection			Availability status		Germplasm collected		Comments 1/
			shoots or branches	culms	seeds	yes	no	field	laboratory	
Oct.17, 85	Bangkok	*Bambusa glaucescens*	/				/			D
Oct.25, 85	Lampang	*B. arundinacea*	/				/			H
		B. vulgaris	/				/			H
		Dendrocalamus membranaceus	/				/			H,D
		Gigantonanthera albociliata	/				/			H
		Thyrsostachys siamensis	/				/			H
		T. oliveri	/				/			H
Nov. 6, 85	Bangkok	*B. glaucescens*	/				/			D
Nov.12, 85		*B. glaucescens*	/				/			D
Nov.25, 85		*B. glaucescens*	/				/			H
Dec. 5, 85		*B. glaucescens*	/				/			H
Dec.11, 85		*B. glaucescens*	/				/			D
Dec.25, 85	Kanjana-buri	*B. brandisii*	/	/		/		/		Do
		B. flexuosa	/	/		/		/	/	Do
		B. pubescens	/	/		/		/		Do
		B. tulda		/			/			D
		B. burmanica		/			/			D
		Cephalostachym virgatum		/		/		/		Do
		D. pergracile	/	/			/			D

Table 8. (Cont'd.)

Date of collection	Location	Species or Clones	Forms of collection			Availability status		Germplasm collected		Comments 1/
			shoots or branches	culms	seeds	yes	no	field	laboratory	
		D. strictus	/	/	/	/		/	/	P
		Gigantochloa hasskarliana	/				/			D
		Melocalamus compactiflorus	/	/		/		/		G
		T. siamensis	/				/			H
		T. siamensis (Rauk Daeng)*/	/	/		/		/		G
Jan.14, 86	Nontaburi	*B. glaucescens*	/			/			/	G
Jan.16, 86		*B. glaucescens*	/			/			/	G
Jan.22, 86	Bangkok	*B. glaucescens*	/				/			H
Feb.27, 86		*B. glaucescens*	/				/			H
Feb. 7, 86		*B. glaucescens*	/				/			D
Feb.10, 86	Nontaburi	*B. glaucescens*	/			/				G
Feb.27, 86		*B. glaucescens*	/			/				G
Mar.12, 86	Nontaburi	*B. glaucescens*	/			/				G
Mar.17, 86		*B. glaucescens*	/			/				G
Mar.20, 86	Bangkok	*B. vulgaris*	/			/			/	G
Apr. 4, 86	Kanjana-buri	*B. arundinacea*	-	-	/	/			/	G,C
		B. burmanica	/	-	-	/			/	G

*/ Local name.

Table 8. (Cont'd.)

Date of collection	Location	Species or Clones	Forms of collection			Availability status		Germplasm collected		Comments 1/
			shoots or branches	culms	seeds	yes	no	field	laboratory	
		D. strictus	/		/	/		/		G
		D. brandisii			/	/			/	G
		O. albociliata	/				/			H
Apr.10, 86	Chiangmai	*Arundinaria suberecta*	/			/			/	G
		B. longispatha	/			/			/	G
		B. ventricosa (clone 1)	/			/			/	G
		B. polymorpha	/			/			/	Do
Apr.11, 86		*T. siamensis* (clone 1)	/			/			/	G
		T. siamensis (clone 2)	/			/			/	G
Apr.12, 86		*B. ventricosa* (clone 2)	/			/			/	H
		B. arundinacea	/			/			/	P
		T. siamensis (clone 3)	/			/			/	Do,C
		T. siamensis (clone 4)	/			/			/	P
Apr.22, 86	Bangkok	*B. vulgaris*	/			/			/	G
		B. vulgaris (Thong Yai) */	/			/			/	G
		B. nana	/			/			/	G
		Phyllostachy sulphurea	/			/			/	Do,C

*/ Local name.

Table 8. (Cont'd.)

Date of collection	Location	Species or Clones	Forms of collection			Availability status		Germplasm collected		Comments 1/
			shoots or branches	culms	seeds	yes	no	field	laboratory	
Apr.30, 86	Nakorn-rachasima (Pakchong)	*B. arundinacea*	/				/			H
		D. asper	/				/			H
		D. asper (Tong Keaw)*/	/			/			/	H,C
		D. asper (Tong Dum) */	/				/			H
		D. membranaceus	/				/			H
		O. allocilliata	/				/			H
		T. siamensis	/				/			H
		T. siamensis (Pakchong)*/	/				/			H
May.2, 86	Bangkok	*T. siamensis*	/				/			H
		Schizostachym blumer	/				/			H
May.9, 86	Prachin-buri	*B. glaucescens* (clone 1)	/			/			/	G
		B. glaucescens (clone 2)	/			/			/	G
		B. longispatha	/				/			D
		B. burmanica	/			/			/	P
		B. burmanica (Leui)*/	/			/			/	Do
		B. nana	/			/			/	G
		D. membranaceus	/				/			D
		D. asper (Tong Moa)*/	/			/			/	G
		T. siamensis (Rauk Daeng)*/	/			/			/	G

*/ Local name.

Table 6. (Cont'd)

Date of collection	Location	Species or Clones	Forms of collection			Availability status		Where was collected		Comments 1/
			shoots or branches	culms	seeds	yes	no	field	laboratory	
May.13,86	Bangkok	*D. giantous*	/				/			H
		P. sulphurea	/				/			H
May.27,86	Bangkok	*B. flexuosa*	/			/			/	G,C
May.30,86	Bangkok	*B. flexuosa*	/			/			/	G,C
		D. giantous	/			/			/	Do

1/ = comments.

C = Callus formation in vitro.

D = All died.

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

G = Growing in vitro or in vivo.

H = Heavy contamination in vitro.

P = Partially died in vitro.

IV. FUTURE WORK PLAN FROM JUNE TO DECEMBER, 1986.

1. TECHNOLOGY DEVELOPMENT.

1.1 Stem-bud segments system.

Experiments will be conducted to study the following outlines:

- continue experiment to improve the survival rate and bud-break efficiency.
- continue experiments to investigate a suitable medium and method for long term culture, multiplication of shoots and improvment of rooting efficiency.
- continue study the effect of low temperature on conservation.
- continue study the effect of growth retardants ie. CCC and daminozide for conservation.
- initiate cryo-preservation experiments for conservation.

1.2 Young leaves culture system.

- continue experiment to investigate a suitable medium and condition for callus multiplication and regeneration.
- continue experiment to investigate the low temperature and growth retardant effect on callus storage.
- initiate the cryo-preservation experiment for long term storage.

1.3 Seeds system.

- repeat experiments on shoot multiplication medium and then apply to other species.
- continue experiment on the effect of low temperature, growth retardant for conservation on other species.

2. GERMPLASM COLLECTION.

- Continue collection of bamboo species in Thailand by expanding the collection areas. Germplasm collection in Sri Lanka, if political atmosphere is improved, will be acheived during this period. Collaboration will then be pursued.

V. CONCLUSION

With some limitations due to unforseen problems, researchers activities have been progressively conducted. Technology development for mass propagation and conservation *in vitro* has been partially developed. Experiments are moving smoothly and we expect to acheive the goal in due time. Germplasm collection in Thailand has been progressing very well, However, germplasm collection in Sri Lanka has not been started and is behind the schedule. If the political atmosphere in Sri Lanka is improved, we'll be able to cope up with the programme soon.