ACTIVITY COMPLETION REPORT

IN VITRO PROPAGATION OF *GONYSTYLUS BANCANUS* (RAMIN) IN SARAWAK

IMPLEMENTING AGENCY

SARAWAK FORESTRY CORPORATION

ACTIVITY COMPLETION REPORT

Activity Identification

a.	**Title**	***In Vitro* Propagation of *Gonystylus bancanus* (Ramin) in Sarawak**
b.	**Implementing Agency**	**Sarawak Forestry Corporation**
c.	**Starting Date**	**October 2012**
d.	**Activity Duration**	**12 months extended for another 7 months without additional funding**
e.	**Activity Cost**	
i.	**ITTO**	**USD 105,000.00**
ii.	**Gov. Contribution**	**USD 87,500.00**
iii.	**Total Cost**	**USD 192,500.00**

ACTIVITY TECHNICAL AND SCIENTIFIC STAFF

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EXECUTIVE SUMMARY

1. ACTIVITY IDENTIFICATION

Gonystylus bancanus (Ramin) is categorized as vulnerable in the IUCN Red List as its populations and habitats have decreased sharply as a result of over-exploitation. In an attempt to curb detrimental population loss of this species, it was listed under Appendix II in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Thus, it is important to improve the productivity of peat swamp forests by planting the degraded areas with Ramin in order to sustain their role as an important source of Ramin timber. On the other hand, the establishment of Ramin plantations in non-peat swamp areas is also crucial to supplement production from the natural peat swamp forests and as a step towards natural forest conservation. With this, raising sufficient planting materials is an important component for both species recovery and plantation establishment purposes.

Planting materials of Ramin can be easily raised through seeds and vegetative propagation. However, under natural conditions, Ramin appears to have irregular flowering and fruiting habits. In addition, Ramin seeds are desiccation intolerant and must be stored at high moisture content. As a result, vegetative propagation plays an important role to prepare large quantity of planting materials to satisfy the need of the Ramin timber industry. Effective protocol to micropropagate Ramin via plant tissue culture techniques must be established. This will enhance the production of Ramin planting materials for reforestation and forest plantation establishment, and as a step towards conservation of natural peat swamp forest.

2. ACTIVITY OBJECTUVES AND IMPLEMENTATION STRATEGY

Specific Objective

The Activity aims to establish effective protocols for *in vitro* propagation of *Gonystylus bancanus* (Ramin) via plant tissue culture techniques (micropropagation) for mass production of this species, ensuring sufficient planting materials to be raised for species recovery programme or Ramin plantation establishment. The objectives of this Activity are (i) to establish effective protocols for axenic (contamination-free) culture establishment of *Gonystylus bancanus* using field-grown materials; and (ii) to establish protocols for *in vitro* regeneration of *Gonystylus bancanus* via direct organogenesis using axenic explants obtained.

Implementation Strategy

The strategies applied for the implementation of this Activity include both technical and scientific aspects as well as managerial aspects. For technical and scientific aspects, the main activities included field visits, collection of planting materials, optimizing surface sterilization to obtain axenic cultures and shoot induction, and data analysis using computer software. The managerial aspect covered the recruitment of a national consultant, national expert, contract research officer and research assistant. Staff was trained on sampling techniques, sample handling, rejuvenation techniques, laboratory experiments and data collection.

3. ACTIVITY PERFORMANCE: DIFFERENCES BETWEEN PLANNED AND REALIZED ACTIVITY IMPLEMENTATION

Specific Objectives: There was no major change in the specific objectives except the additional activities applied in both objective 1 and 2. For objective 1, besides wilding collection, bending of saplings and cuttings was included as an alternative explant source throughout the Activity. Objective 2 aims to establish protocols for *in vitro* propagation of *Gonystylus bancanus* via direct organogenesis using axenic explants obtained. In addition to direct organogenesis, two alternative pathways were applied, i.e. indirect organogenesis and somatic embryogenesis. Details are stated in the technical reports.

Activity Personnel: No amendment.

Time Schedule: Field work was carried out accordingly as per schedule, including phenology monitoring, wildings collection and rejuvenation of plant materials. However, tissue culture work started only in February 2013 instead of October 2012, after the recruitment of a contract research officer and research assistant. The Activity was extended for seven months.

Budget Amendment: No amendment.

4. ACTIVITY ACHIEVEMENTS

Outputs and Specific Objectives Achieved

To achieve the specific objectives, this Activity focused on:

- Field surveys including phenology monitoring, wilding population surveys and sample collection
- Rejuvenation of plant materials, i.e. bending of saplings in the field and cuttings in the green house
- Laboratory experiments including surface sterilization, shoot and callus induction and culture maintenance
- Statistical analysis of data collected

Target Beneficiaries

Propagation protocols developed can be applied for the mass propagation of Ramin to ensure sufficient planting materials be produced in a short time frame for use in species recovery programme and plantation establishment, particularly in Sarawak, Malaysia. Establishment of Ramin plantation and the planting of Ramin in deforested peat swamp forests can supplement the production from the remaining peat swamp forests and as a step towards conservation of natural peat swamp forest. In addition, the protocols developed will also serve as a reference for micropropagation strudy of tropical timber species, particularly *Gonystylus* species.

Preliminary research findings of this Activity were shared with the Center for Forest Biotechnology and Tree Improvement (CBTI) and the Center for Forest Conservation and Rehabilitation in Indonesia on November 2013. These two centers also carried out studies on Ramin propagation and conservation under ITTO-CITES programme.

Two contract staff and a number of Sarawak Forestry Corporation staff were involved in the field activities and laboratory experiments under the guidance of the national consultant and expert engaged. Besides that, local communities were also engaged in the field activities.

5. LESSONS LEARNED

To obtain sufficient and continuous availability of plant materials for laboratory experiments, rejuvenation of plant materials, i.e. bending of saplings in field and cuttings in green house was carried out in a staggered manner.

Work load was higher than as per proposed with the addition of two alternative pathways in objective 2. Coordination and sense of responsibility among staff was crucial in the Activity implementation to ensure the achievement of the Activity objectives according to the schedule.

The difficulties encountered during the implementation were accessibility to flooded-sampling plot during raining season and slow growth of shoot induced due to the plant nature.

6. RECOMMENDATIONS

Based on the research findings, somatic embryogenesis is more promising for *in vitro* propagation of *Gonystylus bancanus* compared to both direct and indirect organogenesis. Thus, this study should be continued for at least another one to two years to obtain more conclusive results. It is also important to share the current findings with CBTI in Indonesia.

PART II – MAIN TEXT

1.0 ACTIVITY IDENTIFICATION

1.1 Activity Content

Social, Economic and Environment

Gonystylus bancanus (Ramin) is the most important source of *Gonystylus* timbers and can be considered as one of the major timbers exported from South-East Asia to countries such as Japan, China, Taiwan and Hong Kong. Ramin timbers are of high demand both locally and internationally. However, Ramin production from Sarawak has declined to the extent that Sarawak needs to import Ramin timbers from other places to cater for the local market demand. This decline in production indicates the reduction of Ramin population in peat swamp forests, putting this species at risk. Ramin population reduction is also associated with land development where forested peatlands are cleared and developed for agriculture, aquaculture, industries and residential uses.

Project Location

Field surveys including phenology monitoring and wildings collection were carried out at three areas in Sarawak, namely the Lingga water catchment area in Sri Aman, Loagan Bunut National Park, Miri as well as Sungai Dijih and Sungai Sebakong, Mukah. Bending of saplings for epicormic shoot induction was carried out in the Lingga water catchment area. Plant materials, such as branches and leaves were collected from both the Lingga water catchment area and Loagan Bunut National Park. Laboratory experiments and cuttings were carried out at the plant tissue culture laboratory of Sarawak Forestry Corporation.

Relevant National and Regional Policies and Programmes

The Sarawak Forest Department's statistics of Ramin exported from the State revealed that the highest volumes exported were in the 1960s and 1970s. However, the production declined to its lowest record in 2000. In order to fulfill the increasing demand of Ramin, Sarawak imported both logs and sawn timbers from Indonesia, Brunei Darussalam and Sabah from 1970 to 1978. Ramin population reduction is also associated with land developments where forested peatlands were cleared and developed for agriculture, aquaculture, industries and residential uses. Thus, in 1980, the Sarawak Government imposed a ban on Ramin logs export as a control measure to protect the Ramin population in peat swamp forests. A further ban of ramin shorts and squares, and restriction of Ramin timber export was implemented in 1991. Four areas of peat swamp forest are classified as totally protected areas including the Loagan Bunut National Park gazetted in 1990 and the Maludam National Park gazetted in 2000.

Gonystylus bancanus is categorized as vulnerable in the IUCN Red List as its populations and habitats have decreased sharply as a result of over exploitation. In an attempt to curb detrimental population loss of this species, it is listed under Appendix II in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

1.2 Origin and Problem

With the increasing demand of Ramin, it is necessary to improve the productivity of peat swamp forests by planting the degraded areas with Ramin in order to sustain their role as an important source of Ramin timber. On the other hand, the establishment of Ramin plantations in non-peat swamp areas is also crucial to supplement production from the natural peat swamp forests and as a step towards natural forest conservation. In this regard, a study was conducted on the planting of Ramin in a non-peat swamp area at the Forest Research Institute Malaysia (FRIM). This 11-year planting trial recorded a survival of 52% with an average total height of 816 cm and a diameter at breast height of 9.1 cm. In order to achieve the two mentioned targets, raising sufficient planting materials is an important component for both species recovery and plantation establishment purposes.

Planting materials of Ramin can be easily raised through seeds and vegetative propagation. However, under the natural conditions, Ramin appears to have irregular flowering and fruiting habits. Seed collection is often very difficult due to poor accessibility to the growing areas of Ramin and can only be carried out around log slide tracks. In addition, Ramin seeds are recalcitrant, and cannot be stored for long period of time. As a result, vegetative propagation plays an important role to prepare large quantity of planting materials to satisfy the need of the Ramin timber industry.

This Activity "*In Vitro* Propagation of *Gonystylus bancanus* (Ramin) in Sarawak" was proposed to establish effective protocols for Ramin propagation using plant tissue culture techniques. To date, only two reports on the preliminary findings of Ramin shoot culture and somatic embryogenesis have been published, but the studies were not conclusive and still in progress.

2.0 ACTIVITY OBJECTIVES AND IMPLEMENTATION STRATEGY

Activity Rationale

Gonystylus bancanus (Ramin) showed strong rooting through stem cuttings and has potential to produce plantlets through tissue culture. The techniques of plant tissue culture give an exponential increase of the propagation coefficient. Depending on the multiplication rate, large volume of plants can be rapidly produced *in vitro* from a relatively few selected source plant. Hence, plant tissue culture techniques can rapidly increase the number of individuals of endangered species with reproductive problems and/or extremely reduced populations due to deforestation.

This Activity aims to develop effective protocols for axenic culture establishment to obtain contamination-free explants through surface sterilization of plant materials. Axenic explants obtained would then be used for direct shoot induction study. Besides that, shoot induction via callus phase would also be included in this Activity as alternative pathway to direct shoot induction.

Specific Objective 1

To establish effective protocols for axenic (contamination-free) culture establishment of *Gonystylus bancanus* using field-grown materials

Specific Objective 2

To establish protocols for *in vitro* regeneration of *Gonystylus bancanus* via direct organogenesis using axenic explants obtained

Activity Implementation Strategy

The activities were as stated below:

1. Identification of national consultant and national experts as collaborator
2. Field survey including phenology monitoring and wildings collection to establish hedge orchard
3. Rejuvenation of plant materials, i.e. bending of saplings to produce epicormic shoots and cuttings to produce axillary shoots
4. Sampling of plant materials, such as epicormic shoots, branches and leaves from the field and axillary shoots from the green house
5. Surface sterilization of plant materials and axenic culture establishment
6. Shoot induction via direct organogenesis, indirect organogenesis and somatic embryogenesis

Risk

The quantity of wildings collected were limited, and there was not sufficient plant materials for tissue culture work throughout the Activity. Thus, bending of saplings in the field and cuttings were carried out to produce juvenile plant materials for the study. Sampling activities encountered difficulties during raining season at the end and beginning of the year as the sampling plots were flooded or inaccessible as a result of big wave in the lake at Loagan Bunut. The response of shoots induced from the explant via direct organogenesis was slow in the culture as expected due to the nature of the plant, i.e. slow growth. Two alternative pathways were studied, namely indirect organogenesis and somatic embryogenesis in addition to direct organogenesis.

3.0 PERFORMANCE OF EACH ACTIVITY

Outputs and Operational Activities	Schedule (Duration)
Output 1.1 Effective protocol for surface sterilization of field-grown plant materials of *Gonystylus bancanus*	
Activity 1.1.1 Planting Stocks collection – wildings will be collected from different populations in peat swamp forests to be kept in green house or used to establish hedge orchard as source of explants. Bending of saplings was conducted in the field to produce epicormic shoots and cuttings were carried out in green house to produce axillary shoots.	October 2012 – September 2013
Activity 1.1.2 Surface sterilization of field-grown materials – explant will go through a series of surface sterilization regime using different disinfectants prior to culturing on media	February 2013 – April 2014
Activity 1.1.3 Data analysis – statistical analysis software, i.e. SPSS will be used to analyze data collected from each experiment	February 2014 – April 2014
Output 1.2 Effective protocol for axenic culture establishment of surface-sterilized explants of *Gonystylus bancanus*	
Activity 1.2.1 Determine the optimum media for axenic culture establishment – surface-sterilized explants will go through pre-treatments before culturing on different culture media to obtain axenic culture	February 2013 – April 2014
Activity 1.2.2 Data analysis – statistical analysis software, i.e. SPSS will be used to analyze data collected from each experiment	February 2014 – April 2014
Output 2.1 Optimum concentrations of cytokinin alone or in combination with auxin for shoot induction of axenic explants obtained	
Activity 2.1.1 Shoots induction - effects of different plant growth regulators, i.e. cytokinins alone and in combinations with auxins at different concentrations to induce shoot formation on contamination-free explants obtained will be studied	February 2013 – April 2014
Activity 2.1.2 Data analysis - number of explants regenerated shoots and number of shoots regenerated per explants will be recorded. Statistical analysis software, i.e. SPSS will be used to analyze data collected from each experiment	February 2014 – April 2014
Output 2.2 Optimum explants for shoot induction	
Activity 2.2.1 Explants for shoot induction - determine the optimum explants for shoot induction, response to treatments applied	February 2013 – January 2014
Activity 2.2.1 Data analysis - statistical analysis software, i.e. SPSS will be used to analyze data collected from each experiment	February 2014 – April 2014
Activity 2.2.3 Dissemination of Activity findings - results obtained will be disseminated through conferences, seminars or publications locally and internationally	November 2013 & November 2014

Activity Achievement

Specific Objective 1, Output 1.1: Effective protocol for surface sterilization of field-grown plant materials of *Gonystylus bancanus*

Ramin wildings were collected from two different locations in Sarawak, i.e. Lingga, Sri Aman and Loagan Bunut National Park, Miri. The collected wildings were initially kept in the nursery for hardening. A trial planting of ten wildings was initiated in the hedge orchard, however the growth rate was very slow. The remaining wildings collected were kept in the green house. The shoot tips, nodes and leaves of the wildings were pruned to be used as source of explants. The growth of these wildings, both in the hedge orchard and green house, was slow and the number of shoots which could be harvested for use was very limited. Thus it is necessary that a method of bud-forcing be adopted. In this study, two methods of bud forcing were applied, i.e. bending of saplings in field and cuttings in green house.

Bending of saplings was carried out in the field in the Lingga water catchment area to induce epicormic shoots formation. Epicormic shoots were detected on the stem of bent saplings after three to four months of bending. A total of 63 bendings was done at different period of time, in a staggered manner in order to ensure sufficient supply of plant materials throughout the study period. After the collection of samples, the epicormic shoots collected were sprayed with 0.2% a.i. Mancozeb and covered with wet newspapers before being brought back to the laboratory for further experimental work.

Plant materials undergone a series of surface-sterilization regimes. For shoot-tip and nodal explants, Clorox treatment was initially applied. However, there was no single axenic explant obtained. Mercuric Chloride ($HgCl_2$) treatments were then examined. Based on the results obtained, the optimum protocols for axenic nodal and shoot-tip explants derived from epicormic shoots were treatments with 0.2% $HgCl_2$ and 0.3% $HgCl_2$ respectively for 5 minutes. For axillary shoots, optimum treatment for axenic shoot-tip explants was 0.2% $HgCl_2$ for 20 minutes. On the other hand, optimum surface sterilization protocol for lamina explants is 0.2% $HgCl_2$ for 10 minutes.

For lamina explants, effectiveness of both Clorox and $HgCl_2$ were also studied at different concentrations and different exposure times. Besides pre-treatment with Clorox and $HgCl_2$, agitation with 0.2% a.i. Mancozeb for one hour or immersion in 60^{o}C warm water for 20 minutes was also studied. However, these pre-treatments did not improve the percentage of axenic explants obtained. The optimum surface sterilization protocol for lamina explants was 0.2% of $HgCl_2$ for 10 minutes.

Specific Objective 1, Output 1.2: Effective protocol for axenic culture establishment of surface-sterilized explants of *Gonystylus bancanus*

Murashige and Skoog (MS), modified MS media and Woody Plant Medium (WPM) were used as the basal media for axenic culture establishment in this activity.

The main problem of axenic culture establishment is fungal and bacterial contamination. The Plant Preservative Mixture (PPM), a biocide, was incorporated into the basal media to inhibit the growth of fungus. After two weeks of culture, the explants were transferred to fresh media of same composition with reduced PPM concentrations. The number of contaminated explants increased after the transfer to lower concentrations of PPM. Thus, as the current practice, after

surface sterilization, explants are cultured on basal medium without the incorporation of biocide or antibiotics.

The percentage of axenic culture obtained increased from time to time with the selected optimum surface sterilization protocols. The highest percentage of axenic shoot-tip explants achieved was 40%, while for nodal explants, the highest percentage recorded was 42%. For lamina explants, the highest percentage obtained was 97%.

Specific Objective 2, Output 2.1: Optimum concentrations of cytokinin alone or in combination with auxin for shoot induction of axenic explants obtained

Axenic explants obtained were transferred to the basal medium incorporated with different cytokines and/or in combination with auxins at different concentrations for shoot induction at three different directions, i.e. direct organogenesis, indirect organogenesis and somatic embryogenesis.

In direct organogenesis, shoot-tip and nodal explants were cultured on MS and modified MS medium added with 6-Benzyl amino purine (BAP) alone and in combination with Napthalene acetic acid (NAA) for shoot induction. Shoot-tip explant sprouted new shoot after one week of culture, while for nodal explant, it took three weeks of culture for new bud to sprout. Generally, only one shoot was induced from each axenic shoot-tip and nodal expant in the culture. One shoot-tip explant sprouted two shoots at 2.5mg/L BAP after 71 days of culture. However, the development of new shoots was very slow. Shoots obtained were further transferred to Indole-3-butyric acid (IBA) for rooting, but no sign of root growth was observed. On the other hand, for lamina explants, effects of 6-benzylamino purine (BAP) in combination with 2,4-Dichlorophenoxyacetic acid (2,4-D) and Napthalene acetic acid (NAA) as well as Thidiazuron (TDZ) alone were studied. BAP in combination with 2,4-D in modified MS medium produced green, globular and compact calli. Globular structures were also detected on the midrib of lamina explants cultured on BAP and NAA in modified MS medium after three months of initiation culture. In general, most of the explants formed calli after two weeks of culture. However, there was no bud or shoot regeneration from the explants.

Based on observation, direct organogenesis showed slow progress due to the plant nature. Thus, two alternative approaches were adopted, i.e. indirect organogenesis and somatic embryogenesis using lamina explants. For indirect organogenesis, 2,4-D was incorporated into MS basal medium for initial callus induction. Calli were detected from lamina explants after two weeks of culture. In order to induce organogenesis, the calli were then transferred to BAP in combination with NAA. The calli turned into green globular structures on the surface, but to date, no new shoot was induced.

For somatic embryogenesis, the somatic embryos formation was detected from calli cultured on WPM medium supplemented with 30, 35 and 40 mg/L NAA. Somatic embryos developed on 30 and 35 mg/L NAA were detected after 84 days of culture, while embryos cultured on 40 mg/L was observed after six months of culture. Somatic embryos formed were isolated and cultured on fresh medium of the same composition for further proliferation. Unfortunately, after nine months of culture, the somatic embryos showed no sign of further proliferation and turned brown eventually.

Specific Objective 2, Output 2.2: Optimum explants for shoot induction

Axillary shoots induced from cuttings in the green house contributed to higher percentage of axenic culture compared to epicormic shoots produced through bending of saplings in the field. This is due to the controlled environment provided for the cuttings in green house such as frequent spraying of cuttings with fungicide and wrapping of shoots induced to avoid direct contact with water from the misting system.

Optimum explants for direct shoot induction are shoot-tip and nodal explants, while for indirect organogenesis and somatic embryogenesis, lamina explants respond better to plant growth regulator for callus induction.

Technical Reports

Three technical reports were produced under this Activity, namely:

1. Axenic Culture Establishment of *Gonystylus bancanus* (Miq.) Kurz (Ramin) in Sarwak
2. Shoot Induction of *Gonystylus bancanus* (Miq.) Kurz (Ramin) in Sarwak
3. Induction of Organogenesis and Somatic Embryogenesis of *Gonystylus bancanus* (Miq.) Kurz (Ramin) in Sarwak

Total Amount of Expenditures and Analysis

From the ITTO contribution of USD 105,000.00, a total of USD 104,571.30 was spent with a balance of USD 428.70 as reflected in the 'Activity Cash Flow Statement – ITTO Contribution' and the 'Activity Financial Statement – ITTO Contribution' in **Annexes 1 and 2** respectively. The contribution from the Government of Malaysia was USD 87,500.00, of which a total of USD 64,057.52 was spent as shown in the 'Activity Cash Flow Statement – Government of Malaysia Contribution' and the 'Activity Financial Statement – Government of Malaysia Contribution' in **Annexes 3 and 4** respectively.

4.0 TARGET BENEFICIARIES INVOLVEMENT

The Activity conducted can serve as a reference or guideline for other researchers with similar interest in other region, including local government, research institutions and private sectors particularly the timber companies. The technical reports produced are also useful for other micropropagation work especially on tropical timber species. The rejuvenation technique applied in the Activity, i.e. bending of saplings, is useful for production of juvenile plant materials for both conventional propagation and tissue culture work for timber species. This technique is not detrimental to the saplings and the number of epicormic shoots produced is much higher compared to coppicing. Sharing of findings with Indonesian researchers was carried out in November 2013 as per report shown in **Annex 5**. Moreover, the Activity carried out also provided extra income for the local community in the activity areas as locals were engaged for the field survey.

5.0 ASSESSMENT AND ANALYSIS

5.1 The Activity design has been efficient in achieving the Activity Objectives and answering the key issues rained in the Activity Documentation

5.2 Implementation strategies which have been taken through technical, scientific and managerial aspects have been effective in the execution of the Activity operational activities and outputs production

5.3 The execution of the Activity has been in accordance with the expectation made during the Activity identification process, the problem to be addressed and the outputs achieved through the implementation approach

5.4 The Activity was implemented as per schedule with the consultation of national consultant and national expert

5.5 The expected outputs achieved in this Activity are crucial for the production of planting materials for species recovery programme and plantation establishment for the conservation of natural peat swamp forests

6.0 LESSONS LEARNED

Activity Development

To ensure the successful implementation and completion of the Activity, cooperation, support and commitment from the consultants and staff involved are required. Local community in the sampling areas also contributed to the success of the Activity, guiding the staff to the right location and assisting in the field works.

Operational Matters

Additional studies were included in order to achieve the specific objectives due to the slow progress in the initial planned-activities.

7.0 CONCLUSION AND RECOMMENDATION

Development Lessons

Though the specific objectives of the Activity were achieved, research findings obtained to date are considered as preliminary and not conclusive. It is recommended that:

- Further study be carried out on *in vitro* propagation of Ramin particularly on somatic embryogenesis
- The Sarawak Government and timber industries to provide continual support on research projects pertaining to the conservation of Ramin
- Timber industries be encouraged to invest in Ramin plantation establishment

Operational Lessons

The Activity Coordinator plays an important role in ensuring successful implementation of the Activity. The support, cooperation and knowledge sharing from the consultants, managers, researchers and staffs of Sarawak Forestry Corporation as well as local people in the sampling areas has contributed to the smooth operation of the activities at both the planning and implementation levels.

8.0 RECOMMENDATION FOR FUTURE PROJECT

Further study of Ramin propagation using plant tissue culture techniques is highly recommended as Micropropagation is the most reliable method for timber species with irregular flowering and fruiting, and recalcitrant seeds. Ramin plant physiology study is also important for better understanding of the species for the propagation study. Technical reports produced will be shared especially with CBTI in Indonesia whose researchers are currently working on micropropagation of Ramin.

Annex 1

ACTIVITY CASH FLOW STATEMENT (in US Dollar)
ITTO CONTRIBUTION

Program Title: ITTO-CITES Program, Phase II

Activity No. : 2 Period covered (ending on): April 2014

Activity Title: *In Vitro* Propagation of *Gonystylus bancanus* (Ramin) in Sarawak.

Component	Reference	Date	Amount	
			in US$	Local Currency In RM
A. Funds received from ITTO:				
1. First instalment		Nov-12	52,500.00	162,225.00
2. Second Instalment		Oct-13	52,500.00	162,225.00
3. Third instalment				
4. Fourth instalment				
5. Interest on bank deposits				
Total Funds Received:			105,000.00	324,450.00
B. Expenditures (by Executing Agency):				
10 Personnel				
11. Coordinator				
12. Other Personnel				
12.1 Assistant 1: Research Officer			13,883.93	42,901.34
12.2 Assistant 1: Research Assistant			7,283.90	22,507.25
12.5 Other labour				
13. National Experts				
13.1 Expert 1			2,849.90	8,806.19
13.2 Expert 2			21,844.66	67,500.00
13.3 Expert 3				
14. International Consultant(s)				
14.1 Consultant 1				
14.2 Consultant 2				
15 Personnel Total:			45,862.39	141,714.78
16. Workshop/Seminar and Training (specify beneficiaries)				
16.1 Travel/Transportation Costs (participants)				
16.2 Daily Subsistence Allowances (participants)				
16.3 Venue and Logistics				
16.4 Workshop Materials				
16.5 Others				
17. Workshop/Seminar and Training Total:			0.00	0.00

Annex 1

ACTIVITY CASH FLOW STATEMENT (in US Dollar)
Continued

Component	Reference	Date	Amount	
			in US$	Local Currency in RM
20. Sub-contracts				
21. Sub-contract(Topic e.g. mapping, etc.)				
22. Sub-contract (Topic 2)				
29. Sub-contracts Total:			0.00	0.00
30. Travel				
31. Daily Subsistence Allowance				
31.1 National Expert(s)				
31.2 International Consultant(s)				
31.3 Others			9,068.07	28,020.34
32. International Travel				
32.1 National Expert(s)				
32.2 International Consultant(s)				
32.3 Others				
33. Local Transport Costs				
33.1 National Expert(s)			491.91	1,520.00
33.2 International Consultant(s)			1,093.53	3,379.01
33.3 Others				
39. Travel Total:			10,653.51	32,919.35
40. Capital Items				
41. Premises				
42 Vehicle(s)				
43 Capital Equipment				
43.1 Computer Equipment (specify)				
43.2 Others (specify)				
49. Capital Items Total:			0.00	0.00
50. Consumable Items				
51. Raw materials			43,773.11	135,258.91
52. Spares				
53. Utilities			1,171.18	3,618.95
54. Office Supplies				
59. Consumable Items Total:			44,944.29	138,877.86

Annex 1

ACTIVITY CASH FLOW STATEMENT (in US Dollar)
Continued

Component	Reference	Date	Amount	
			in US$	Local Currency in RM
60. Miscellaneous 61. Sundry 62. Contingencies			3,111.12	9,613.36
69. Miscellaneous Total:			3,111.12	9,613.36
70. Others (specify) 71. Others (specify)				
79. Others Total:			0.00	0.00
Total Expenditures To-date:			104,571.31	323,125.35
Remaining Balance of Funds (A-B):			428.69	1,324.65

Exchange rate: 1USD = RM3.0903

Notes: (1) Amounts in U.S. dollars are converted using the average rate of exchange when funds were received by the Executing Agency;

(2) Amount of expenditures in US dollar should be the same as amount shown in column (c) of the Financial Statement (with direct link from the Cash Flow Statement);

(3) Provide a **list of all expenditure components** (listing the expenditures on excel format, showing date, payee, category/components of expenditures and the amount, both in local currency and in US dollar);

(4) Submit all **actual supporting payment documents/evidences** (filed in the same sequence as the entries in the list of expenditures in (3) above); and

(5) Submit **bank reconciliation statements** along with the bank statement to support the remaining balances/funds in the Cash Flow Statement.

Annex 2

ACTIVITY FINANCIAL STATEMENT (in US Dollar)
ITTO CONTRIBUTION

Program Title: ITTO-CITES Program, Phase II
Activity No.:2 Period covered (ending on): April 2014
Activity Title:*In Vitro* Propagation of *Gonystylus bancanus* (Ramin) in Sarawak.

Component	Original Amount (A)	Expenditures To-date			Available Funds (E) {A - D}
		Accrued (B) b/	Expended (C)	Total (D) {B + C}	
I. Funds managed by Executing Agency					
10. Personnel					
11. Coordinator					
12. Other Personnel	21,170.00				2.17
12.1 Assistant 1: Research Officer			13,883.93	13,883.93	
12.2 Assistant 2: Research Assistant			7,283.90	7,283.90	
12.5 Other labour					
13. National Experts					
13.1 Expert 1	2,849.90		2,849.90	2,849.90	0.00
13.2 Expert 2	21,845.10		21,844.66	21,844.66	0.44
13.3 Expert 3					
14. International Consultant(s)					
14.1 Consultant 1					
14.2 Consultant 2					
15. Personnel Total:	45,865.00	0.00	45,862.39	45,862.39	2.61
16. Workshop/Seminar and Training (specify beneficiaries)					
16.1 Travel/Transportation (participants)					
16.2 Daily Subsistence Allowance (participants)					
16.3 Venue and Logistics					
16.4 Workshop Materials					
16.5 Others					
17. Workshop/Seminar and Training Total:	0.00	0.00	0.00	0.00	0.00
20. Sub-contracts					
21. Sub-contract					
22. Sub-contract					
29. Component Total:	0.00	0.00	0.00	0.00	0.00

Annex 2

ACTIVITY FINANCIAL STATEMENT (in US Dollar)
Continued

Component	Original Amount (A)	Expenditures To-date			Available Funds (E) { A - D }
		Accrued (B) b/	Expended (C)	Total (D) { B + C }	
30. Travel	10763.00				109.49
31. Daily Subsistence Allowance					
31.1 National Expert(s)					
31.2 International Consultant(s)					
31.3 Others			9,068.07	9,068.07	
32. International Travel					
32.1 National Expert(s)					
32.2 International Consultant(s)					
32.3 Others					
33. Local Transport Costs					
33.1 National Expert(s)			491.91	491.91	
33.2 International Consultant(s)					
33.3 Others			1,093.53	1,093.53	
39. Travel Total:	10,763.00	0.00	10,653.51	10,653.51	109.49
40. Capital Items					
41. Premises					
42. Vehicle(s)					
43. Capital Equipment					
43.1 Computer Equipment (specify)					
43.2 Others					
49. Capital Items Total:	0.00	0.00	0.00	0.00	0.00
50. Consumable Items					
51. Raw Materials	44000.00		43,773.11	43,773.11	226.98
52. Spares					
53. Utilities	1,172.00		1,171.18	1,171.18	0.82
54. Office Supplies					
59. Consumable Items Total:	45,172.00	0.00	44,944.29	44,944.29	227.71

Annex 2

ACTIVITY FINANCIAL STATEMENT (in US Dollar)
Continued

Component	Original Amount (A)	Expenditures To-date			Available Funds (E) {A - D}
		Accrued (B) b/	Expended (C)	Total (D) {B + C}	
60. Miscellaneous 61. Sundry 62. Contingencies	3,200.00		3,111.12	£3,111.12	£88.88
69. Miscellaneous Total:	3200.00	0.00	3111.12	3111.12	88.88
70. Others (specify) 71. Others (specify) Executing Agency Management Cost					
79. Others Total	0.00	0.00	0.00	0.00	0.00
100. GRAND TOTAL:	105,000.00	0.00	104,571.31	104,571.31	428.69

Exchange rate: 1USD = RM3.0903

Note: Budget Components are those detailed in the Activity Document.

a/ The **Cash Flow Statement must be completed first**, before the other inputs into this Financial Statement;

b/ Accured expenditure: expenditures incurred during the reporting period, but not yet settled

c/ Amounts under the "Expended" column will be transferred automatically from the Cash Flow Statement (with direct link); and

d/ Refer to the notes in the Cash Flow Statement for the supporting **information and documents** that are to be submitted to the ITTO Secretariat.

Annex 3

ACTIVITY CASH FLOW STATEMENT (in US Dollar)
GOVERNMENT OF MALAYSIA CONTRIBUTION

Program Title: ITTO-CITES Program, Phase II
Activity No. : 2 — Period covered (ending on): April 2014
Activity Title: *In Vitro* Propagation of *Gonystylus bancanus* (Ramin) in Sarawak.

Component	Reference	Date	Amount	
			In US$	Local Currency In RM
A. **Funds received from GoM:**				
1. First instalment		Nov-12	43,750.00	135,187.50
2. Second instalment		Oct-13	43,750.00	135,187.50
3. Third instalment				
4. Fourth instalment				
5. Interest on bank deposits				
Total Funds Received:			87,500.00	270,375.00
B. **Expenditures (by Executing Agency):**				
10 Personnel				
11. Coordinator				
12. Other Personnel				
12.1 Activity Leader			13,150.81	40,636.00
12.2 Assistant 1			8,737.86	26,999.99
12.3 Assistant 2			7,844.18	24,238.52
12.4 Assistant 3			8,447.57	26,102.99
12.5 Other labour			4,906.22	15,160.22
13. National Experts				
13.1 Expert 1				
13.2 Expert 2				
13.3 Expert 3				
14. International Consultant(s)				
14.1 Consultant 1				
14.2 Consultant 2				
15 Personnel Total:			43,086.64	133,137.72
16. Workshop/Seminar and Training (specify beneficiaries)				
16.1 Travel/Transportation Costs (participants)				
16.2 Daily Subsistence Allowances (participants)				
16.3 Venue and Logistics				
16.4 Workshop Materials				
16.5 Others				
17. Workshop/Seminar and Training Total:			0.00	0.00

Annex 3

ACTIVITY CASH FLOW STATEMENT (in US Dollar)
Continued

Component	Reference	Date	Amount	
			in US$	Local Currency in RM
20. Sub-contracts				
21. Sub-contract(Topic e.g. mapping, etc.)				
22. Sub-contract (Topic 2)				
29. Sub-contracts Total:			0.00	0.00
30. Travel				
31. Daily Subsistence Allowance				
31.1 National Expert(s)				
31.2 International Consultant(s)				
31.3 Others			3,009.71	9,300.00
32. International Travel				
32.1 National Expert(s)				
32.2 International Consultant(s)				
32.3 Others				
33. Local Transport Costs				
33.1 National Expert(s)				
33.2 International Consultant(s)				
33.3 Others				
39. Travel Total:			3,009.71	9,300.00
40. Capital Items				
41. Premises				
42 Vehicle(s)				
43 Capital Equipment				
43.1 Computer Equipment (specify)				
43.2 Others (specify)				
49. Capital Items Total:			0.00	0.00
50. Consumable Items				
51. Raw materials			11,650.49	36,000.01
52. Spares				
53. Utilities			5,825.24	17,999.99
54. Office Supplies			485.44	1,500.01
59. Consumable Items Total:			17,961.17	55,500.02

Annex 3

ACTIVITY CASH FLOW STATEMENT (in US Dollar)
Continued

Component	Reference	Date	Amount	
			in US$	Local Currency in RM
60. Miscellaneous 61. Sundry 62. Contingencies				
69. Miscellaneous Total:			0.00	0.00
70. Others (specify) 71. Others (specify)				
79. Others Total:			0.00	0.00
Total Expenditures To-date:			64,057.52	197,937.74
Remaining Balance of Funds (A-B):			23,442.48	72,437.26

Exchange rate: 1USD = RM3.0903

Notes: (1) Amounts in U.S. dollars are converted using the average rate of exchange when funds were received by the Executing Agency;

(2) Amount of expenditures in US dollar should be the same as amount shown in column (c) of the Financial Statement (with direct link from the Cash Flow Statement);

(3) Provide a **list of all expenditure components** (listing the expenditures on excel format, showing date, payee, category/components of expenditures and the amount, both in local currency and in US dollar);

(4) Submit all **actual supporting payment documents/evidences** (filed in the same sequence as the entries in the list of expenditures in (3) above); and

(5) Submit **bank reconciliation statements** along with the bank statement to support the remaining balances/funds in the Cash Flow Statement.

Annex 4

ACTIVITY FINANCIAL STATEMENT (in US Dollar)
GOVERNMENT OF MALAYSIA CONTRIBUTION

Program Title: ITTO-CITES Program, Phase II
Activity No.:2 Period covered (ending on): April 2014
Activity Title:*In Vitro* Propagation of *Gonystylus bancanus* (Ramin) in Sarawak.

Component	Original Amount (A)	Expenditures To-date			Available Funds (E) { A - D }
		Accrued (B) b/	Expended (C)	Total (D) { B + C }	
I. Funds managed by Executing Agency					
10. Personnel	45,000.00				1,913.36
11. Coordinator					
12. Other Personnel					
12.1 Activity Leader			13,150.81	13,150.81	
12.2 Assistant 1			8,737.86	8,737.86	
12.3 Assistant 2			7,844.18	7,844.18	
12.4 Assistant 3			8,447.57	8,447.57	
12.5 Other labour			4,906.22	4,906.22	
13. National Experts					
13.1 Expert 1					
13.2 Expert 2					
13.3 Expert 3					
14. International Consultant(s)					
14.1 Consultant 1					
14.2 Consultant 2					
15. Personnel Total:	45,000.00	0.00	43,086.64	43,086.64	1,913.36
16. Workshop/Seminar and Training (specify beneficiaries)					
16.1 Travel/Transportation (participants)					
16.2 Daily Subsistence Allowances (participants)					
16.3 Venue and Logistics					
16.4 Workshop Materials					
16.5 Others					
17. Workshop/Seminar and Training Total:	0.00	0.00	0.00	0.00	0.00
20. Sub-contracts					
21. Sub-contract (Topic e.g. mapping, etc.)					
22. Sub-contract (Topic 2)					
29. Component Total:	0.00	0.00	0.00	0.00	0.00

Annex 4

ACTIVITY FINANCIAL STATEMENT (in US Dollar)
Continued

Component	Original Amount (A)	Expenditures To-date			Available Funds (E) { A - D }
		Accrued (B) b/	Expended (C)	Total (D) { B + C }	
30. Travel 31. Daily Subsistence Allowance 31.1 National Expert(s) 31.2 International Consultant(s) 31.3 Others 32. International Travel 32.1 National Expert(s) 32.2 International Consultant(s) 32.3 Others 33. Local Transport Costs 33.1 National Expert(s) 33.2 International Consultant(s) 33.3 Others	4,000.00		3,009.71	3,009.71	990.29
39. Travel Total:	4,000.00	0.00	3,009.71	3,009.71	990.29
40. Capital Items 41. Premises 42. Vehicle(s) 43. Capital Equipment 43.1 Computer Equipment (specify) 43.2 Others					
49. Capital Items Total:	0.00	0.00	0.00	0.00	0.00
50. Consumable Items 51. Raw Materials 52. Spares 53. Utilities 54. Office Supplies	31,000.00		11,650.49 5,825.24 485.44	11,650.49 5,825.24 485.44	
59. Consumable Items Total:	31,000.00	0.00	17,961.17	17,961.17	13,038.83

Annex 4

ACTIVITY FINANCIAL STATEMENT (in US Dollar)
Continued

Component	Original Amount (A)	Expenditures To-date			Available Funds (E) { A - D }
		Accrued (B) b/	Expended (C)	Total (D) { B + C }	
60. Miscellaneous 61. Sundry 62. Contingencies					
69. Miscellaneous Total:	0.00	0.00	0.00	0.00	0.00
70. Others (specify) 71. Others (specify) Executing Agency Management Cost	5,000.00	0.00	0.00	0.00	5000.00
79. Others Total	5,000.00	0.00	0.00	0.00	5000.00
100. GRAND TOTAL:	85,000.00	0.00	64,057.52	64,057.52	20,942.48

Exchange rate: 1USD = RM3.0903

Note: Budget Components are those detailed in the Activity Document.

a/ The **Cash Flow Statement must be completed first**, before the other inputs into this Financial Statement;

b/ Accrued expenditure: expenditures incurred during the reporting period, but not yet settled;

c/ Amounts under the "Expended" column will be transferred automatically from the Cash Flow Statement (with direct link); and

d/ Refer to the notes in the Cash Flow Statement for the supporting **information and documents** that are to be submitted to the ITTO Secretariat.

Annex 5

Study Visit to the Center for Forest Biotechnology and Tree Improvement, Yogyakarta, Indonesia
(Activity 2: *In Vitro* Propagation of *Gonystylus bancanus* (Ramin) in Sarawak)

Date : 6th to 9th November 2013

Location : Center for Forest Biotechnology and Tree Improvement Research
Forestry Research and Development Agency
Ministry of Forestry, Yogyakarta, Indonesia

Objective : To exchange information on stem cuttings and *in vitro* propagation of Ramin from the research team in Indonesia, and to share our preliminary findings on the *in vitro* propagation of Ramin with them

Itinerary :

06.11.2013	Departed Kuching for Yogyakarta via Kuala Lumpur
07.11.2013	Visited the Center for Forest Biotechnology and Tree Improvement (CBTI)
	Briefing and discussion with the Director of CBTI and his research team
	Presentation on *in vitro* propagation of ramin and rooted cuttings technique by Sarawak
08.11.2013	Visited the Plant Tissue Culture Laboratory, CBTI
09.11.2013	Departed Yogyakarta for Kuala Lumpur
10.11.2013	Departed Kuala Lumpur for Kuching

Work Done

The Center for Forest Biotechnology and Tree Improvement (CBTI) was established in 1992.The Center focuses on tree improvement research for indigenous species, covering areas such as conservation and rehabilitation, forest productivity, forest engineering and product processing as well as climate change and forest policy. Their main task is to produce genetically improved seeds to enhance productivity and quality of forest plantations. There are six different laboratories at the Center, i.e. molecular lab, tissue culture lab, seed and reproduction lab, pest and disease labs, and oil analysis lab. Some of the labs and study plots are located at a distance away from the Center.

CBTI carried out the study on *in vitro* propagation of Ramin for four (4) months in 2008 and another five (5) months in 2010 under the ITTO CITES programme. They are currently working on the same project in collaboration with a private company. The Activity leader for this project is Madam Yelnititis. She explained briefly on her past research on this species. Two approaches were applied, i.e. axillary shoots induction and somatic embryogenesis. To date, for axillary shoots induction, no plantlet was produced. Murashige and Skoog (MS) medium supplemented with 6-benzylamino purine (BAP) was used as basal medium. Bud break was observed after 8 weeks of culture. However, new shoots obtained turned brown and eventually died after a long period in culture without any rooting. On the other hand, for somatic embryogenesis, viable calliwere obtained from leaf and shoot-tip explants. Auxin 2,4-D was applied to induce embryogenic callus. These results were similar to those obtained in our current Activity carried out in Sarawak.

Mr. Tajudin EdyKomar from the Center for Forest Conservation and Rehabilitation also briefed us on the Activity on Ramin cuttings conducted at his Centre under the ITTO CITES programme. The project started in 2005 with the aim of rescuing this species from non-status land and its conservation. They have established a 20-hectare Ramin trial planting plot in South Sumatra consisting of planted Ramin wildings and cuttings. Hedge plants were coppiced to produce young shoots as a source of planting materials for the cuttings. Each coppiced hedge plant produced an average of one to two young shoots. A KOFFCO system where the green house is equipped with a misting and fogging system was designed and established for the cuttings. Mr. Tajudin further added that this system was not cost effective and thus, would not be feasible for the mass production of ramin cuttings.

Conclusion

For direct organogenesis and somatic embryogenesis, similar results were obtained in Sarawak as presented by the plant tissue culturist of CBTI. The common problem encountered is the nature of the plant. Though new shoots and calli were successfully induced, the growth development was slow. Understanding of the plant physiology during this growth stage for this species may enhance the methodologies for both the conventional and *in vitro* propagation of the species.

Discussion with Dr Amir Wardhana, the Director of CBTI and Mr. Tajudin Edy Komar, the National Programme Coordinator for ITTO CITES FORDA Phase 2.

Ramin calli in plant tissue culture at CBTI


Figures 2 & 3.Presentation of mementos to Dr. Amir Wardhana and Mr.Tajudin Edy Komar

Figure 4.Presentation of memento to Sarawak from Dr. Amir Wardhana

Acknowledgement

I would like to thank Mr Thang Hooi Chiew for recommending this visit to CBTI and to share our findings with them. I am also grateful to ITTO for the approval to undertake the visit in Indonesia.

Report by Linna Chieng

10 January 2014

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