FINAL REPORT

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IN VITRO CONSERVATION AND PROPAGATION OF THREE ECONOMIC SPECIES OF RATTANS

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EXECUTIVE SUMMERY

Research on rattans tissue culture in Thailand had been conducted since May 1988 at The Royal Chitralada Projects and Dept. of Horticulture, Kasetsart University. Rattans are spiny climbing palms that are found in South East Asia and neighboring regions. The utilization of rattans have been known since ancient time such as weaving materials, basket wares, matting, furnitures etc. At present the demand for rattan canes has been increasing year after and overexplotiation of canes from natural forest caused the depletion of natural rattan resources, extinction may be end result. Conservation and propagation of rattans must be viewed as urgent. One of the major constraints in embarking on rattan plantation is the INSUFFICIENT AVAILABILITY OF RATTAN SEEDS.Tissue culture would be accelerate planting stock production to meet the increasing demand for large scale plantation.

The purpose to develop in vitro conservation and propagation techniques of three economic species,: Wai Nam Pueng (Calamus sp.), Wai Takra Thong (Calamus caesius Blume.) and Wai Hom (Calamus pandanosmus Furtado); to promote the existing germplasm collection under the Royal Rattan Development Project. Tissue culture technological development has been successful in propagation. Conservation aspect by using low temperature growth retardant, growth inhibitors. Germplasm collection in the laboratories and in the fields were established.

Tissue culture of Wai Nam Pueng (Calamus sp.), Wai Takra Thong Calamus caesius Blume.) and Wai Hom (Calamus pandanosmus Furtado); were studied by using modified Murashige and Skoog (1962) (MS)medium, Woody Plant medium (WPM) and Y3 medium with various concentration of auxin (2,4-D, picloram, IBA, NAA) and cytokinin (BAP, kinetin, 2iP). It founded that embryo culture of Calamus sp. and Calamus caesius Blume. grew well and gave plantlets on MS medium added with 0.5 mg/l 2,4-D + 2 mg/l BAP. Callus of Calamus sp. developed to be embryoids further developed to shoots during 3-4 months. Shoots of Calamus sp. gave 90 % of roots after cultured for 2 months on MS medium added with 0.5 mg/l NAA or 8 mg/l IBA. Culture of young leaves and young petiole of Calamus sp. on MS medium added with 500 mg/l casein hydrolysate plus different concentration of 2,4-D and BAP in dark conditions for 60 days, the result showed that callus produce from young leaf while use 5-40 mg/l 2,4-D. Culturing young leaf of Calamus caesius Blume. on MS medium 10-80 mg/l 2,4-D obtain callus formation. Great callus formation from young petiole of Calamus sp. was observed on MS medium added with 500 mg/l casein hydro lysate and added with 2 mg/l BAP + 6 mg/l /2,4-D. Callus differentiaed to be embryoids with in 30 days and embryoids could form directly from young petiole for 90-120 days.

The experiments on callus induction, multiplication and differentiation of Calamus caesius were conducted on various media added with 2,4-D and BAP, the result showed the explant from embryogenic tissue on MS medium could be developed into good callus formation. The growth were better than cultured on Y3 and WPM media. Callus induction on MS media additional with various combination and concentration of 2,4-D, IBA NAA, picloram, BAP, 2iP and kinetin. The results showed that in every combination it could induce callus and the combinations of 2,4-D + BAP and IBA+BAP were selected. Effect of picloram was as same as 2,4-D but

the concentration of picloram was less than ten times. Callus induction of embryogenic tissue of Calamus caesius cultured on MS media added with picloram under light condition had been better than under the dark condition. Callus formation was good but decline on growth as increasing concentration of picloram and duration. The studying on multiplication of Calamus caesius callus under different conditions appeared that they could multiple at 30°C under the dark 24 hr-daily on MS medium added with 0.5 mg/l 2,4-D + 2 mg/l BAP was the best. The development of callus on MS medium with 2,4-D and BAP under 16 hr-daily light condition showed better result on callus enlargement and shoot formation than under the dark. Callus development to be embryogenesis and shoot formation in 45 days.

Callus induction of Calamus caesius in vitro seedling, employed explant from various parts such as cabbage, stem, root tip,young leaf and petiole appeared that the explant from cabbage obtained organogenesis and developed to be 6-10 shoots/vessel. Root formation were obtained 100 % for one month on MS medium devoid of plant growth regulator and transplanting in the nursery were successful. Clonal selection of Calamus caesius through tissue culture were conducted. The various clones were different on growth, root formation and transplanting.

Conservation aspects were conducted and studied effect of low temperature on rattan multiple shoots, in vitro seedling and embryoids. The multiple shoots of Calamus caesius incubated at 5°C and 10°C turned brown and die in one week. Seedling could survive and growth very slow at 5°C and 10°C. In vitro seedlings were effected by growth retardants, growth inhibitors and Mixed media. The seedlings which cultured on MS media added with 0.2 % activated charcoal and devoid of plant growth regulator for 6- 24 months could induced multiple shoots and regenerated to be plantlets.

Rattan seedlings were collected from the natural forest and planted in the nursery of the Royal Chitralada Projects and Dept. of Forestbiology, Faculty of Forestry, Kasetsart University. Collection by planting in the Diperocarps Demonstrated Forest on Chitralada Palace ground. The collection and planting rattans at the Royal Development Study Center in Cheingmai province obtained good result.

The encoragement for interesting parties in conservation all kinds of rattan species by receiving the official co-operation under the leading of Trang's govornor and the district council together with the rural villagers in joining together protecting the collection of rattan species.

From supporting of USAID for this project produce the impact which considered to had a royal initiation of the Crown Princess on Plant Genetic Conservation Projects. Rattan Conservation was an activity which transfer technology from rattan research work to extend and cooperation with the government agencies, universities and other institutes. The Royal Chitralada Projects by The Plant Genetic Conservation Project will be an exist and encorage future rattan reserach and development which have the priorities such as : Survey existing resources, Germplasm collection, development of propagation techniques,Investigation of technologies for plantation cultivation, evaluation of domestic use, improved harvesting system, use and marketing.

Now the Plant Tissue Culture Laboratory, The Royal Chitralada Projects and Plant Genetic Conservation Project as a Royal initiation of the Crown Princess MahaChakri Sirindhorn has the collaboration with other government agencies and laboratories for surveying, collecting and studying on survey existing resources of rattans. Studying on development of nutrient formula and appropiate media for Rattan Tissue Culture, Culti vation, cultural practice and management of rattan plantation. Studying on Rattan Identification through Electrophoresis techniques, cell suspension culture and regeneration of rattan and studying on nutrition of Edible Rattans.

BACKGROUND AND INTRODUCTION

The government of the United States of America action through the Agency for International Development (AID) is funding a research project entitled " IN VITRO CONSERVATION AND PROPAGATION OF THREE ECONOMIC SPECIES OF RATTANS " by means of a grant of US$ 150,000 to the Royal Thai Government. The effective period of this grant is May 16,1988 to December 31, 1991 and extend period of the project to November 30, 1992. It has been agreed that The Royal Chitralada Project, Bureau of the Royal Household administer the grant on behalf of the Department of Technical and Economic Cooperation.

RESEARCH OBJECTIVES

The ultimate objectives of this project is to develop a new technology by using tissue culture techniques for rattan conservation and mass propagation. This technology will be relevance to worldwide conservation of rattan, especially those which are economically important and the technology developed will be feasible for mass propagation in order to serve for the increasing demand of rattan for household industry. The stated objectives are :

1. *to develop in vitro conservation techniques for three economic species of rattans: Wai Nam Pueng (Calamus sp.), Wai Takra Thong (Calamus caesius Blume.) and Wai Hom (Calamus pandanosmus Furtado);*
2. *to develop plant tissue culture techniques for mass propagation of these three species; and*
3. *to collect and conserve these three species by in vitro culture to augment the existing germplasm collection under the Royal Rattan Development Project.*

What problem was addressed :

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

Why the problem important to development :

Rattan is spiny climbing plams belonging to the Lepidocaryoid, a major group of the Palm family (Moore,1973). Ten genera are found in the South East Asia and neighbouing regions. Calamus, Daemonorops, Korthalsia, Plectocomia, Myrialepsis, Calospatha, Ceratolobus, (Dransfield, 1979) Pogonotium (Dransfield, 1980a), and Retispata (Dransfield, 1980b) . Aside from their occurence in the Malay Peninsular, Sumatra, Java, Borneo, Selawesi, New Guinea, Fiji, the Philippines, Thailand, the northeastern part of the Indian subcontinent, and China,

rattan are also found in West Africa (Moore,1973) In all, the species of rattan around the world may possibly number about 600 species (Dransfield, 1979). There are about 50 species of rattans in Thailand, in 6 genera, viz Calamus (the largest genus), Daemonorops, Korthalsia, Plectocomia, Plectocomiopsis, and Myrialepsis. Rattans are found mainly in Southern part of Thailand, Where more than 40 species have been reported as growing in moist evergreen forests from the lower plains to mountain tops (Vongkalung, 1985).

The utilization of rattan have been known since ancient time for numeral purposes which may be classified in to three classes, namely for cordage, furniture and miscellaneous such as, basket ware, matting, agricultural tools, the other household functional and decorative items etc. They are utilized by indiginous people for their daily use as well as in the ceremonial ritual. In addition, it is used for food, condiments or seasoning and medicine, but the most important is being use in furniture industry. At present rattan becomes an industrial and exporting crops of many developing countries. It is obvious that turning rattan into various manufactured items would increased incomes as well as creating more employment in the rural areas. The steadily increasing demand of rattan cane for rattan industries lead to continuous exploitation of rattan from the natural forest and caused depletion of the rattan resources. The overexploitation and deliberate change in the forest ecosystem are also responsible for the loss of rattan flora because of their susceptibility to the environmental changes. With the drastic reduction in tropical forest, the natural supply of this important forest product is also drastically diminishing in South East Asia. So it is neccessary to establish the rattan plantation in the selected areas rather than to rely on the natural supply of rattan from the forest Large scale plantation will ensure that the rattan trade can be sustained and at the same time it will be gauranteed, for the conservation of those economic rattan species. Nevertheless, problems are also related to the supply of sufficient planting materials, uniformity of planting stocks and sources of good germplasms. So mass propagation of rattan must now be viewed as an urgent matters. Researches have already been initiated in Thailand, the Philippines, Malaysia, Indonesia and India, mainly togather silviculture information from cultivation trials of commercially valuable species (Menon,1980). Rattan research with special emphasize on germplasm conservation has been partly done in Thailand and elsewhere such as in Malaysia, the Philippines. However works have been done on field collection and collection and conservation. In Thailand, the Royal Forest Department has planted rattans (mainly C.caesius) at I-Sa-Tia natural forest, Narathiwat province, over and area of 199 ha. planted since 1980 (Vongkalung, 1985). Malaysia was conducted rattan plantation in 1975, the rattan research programmes formally initiated by the Forest Research Institute Malaysia at Kepong, Selangor. The project started with a silviculture programme concentrating on three species of economic importance, viz. Calamus manan, Calamus caesius and Calamus spipionium (Wong and Manokaran,1985). In the Philippines was established rattan gene bank to conserve the dwindling resource of very important forest commodity that will be a source of planting material. Plantation was started in 1984 with a totalof 444 plants in 30 taxa (Lapis, 1986).

The nature of very low seed viability and germinating ability coupled with the long dormant period from sowing to germination, have

resulted in the dwindling supply of planting materials in the nature. Germination rate of certain species of Calamus have been reported to vary from 3-80 % depending on the species and media used (Generlao, 1977) and onset of germination took about 1-3 months as recorded from a study of 12 species of Calamus, 14 species of Daemonorops, 3 species of Korthalsia and 3 of minor species, that the germination rate also varied due to genetic differences and the degree of repiness of rattan fruits at harves ting time. The main problem facing the cultivators is the availability of seed supply which due to the fact that fruits can only be obtained from mother plants in remote and in certain accessible areas. Seeds should be collected witle they are still on the tree because the fallen fruits are proved to be deteriorated by fungal infection. Not only that, rattan stem is thorny, so it is difficult to collect the seeds (Aziah, 1985). Another problem is that the limited supply of seeds is also consumed by monkey, birds and even squirrels. Another limitation is observed in Calamus which includes species that are mostly important for commercial application is dioecious plant which fruits and progeny are produced only by the female plants. Therefore, only a proportion of mature plants in any area will bear fruits (Aziah , 1985 ; Umali-garcia, 1985). The limitation of germination rate could partially be overcome by using wildings or suckers. Vegetative propagation of rattan by the conventional method is limited by morphological characteristic such as single solitary stem and slow growing which are difficult for vegetative propagation. In general, for clustering rattan which produce suckers by development of rhizomes they can propagate by division of sucker but the process is rather slow and the survival rate also very low. Apart from that, the solitary stem which posses a single apex, is not possible to multiply by the conventional vegetative propagation such as cutting, grafting and layering.

As mentioned earlier, there are some successful works in developing rattan plantlets via in vitro, such as those reported in Malaysia, Indonesia and the Philippines. So it is quite clear that tissue culture could serve as a tool for conservation and propagation. However, the works have been carried out only in the laboratory as the basic tissue culture propagation and none of them are successfull in perfection of methodology for mass propagation. It is neccessary to look further for other species of rattan which have a great potential for commercial plantation.

Therefore, tissue culture could be a possible solution to the mass propagation of rattan for both clustering and solitary types. The tissue culturists in Malaysia are well aware of the success and drawbacks encountered in case of oil palm. complete plantlet of Calamus manan was produced in vitro (Aziah, 1985) from callus originating from mature embryo and the cycle of regeneration was completed and in vitro production of plantlets of Calamus manan appears to be feasible (Gunawan,1986). In Aziah's report, percentage of success in transplanting to soil is low because of the hardening procedure which is the most crucial part of the experiment. In the Philippines experiment via in vitro works are also con ducted on some rattan species using either embryo or the shoot meristem and the regeneration of callus tissue resulted in shoot and plantlet formation (Umali- Garcia, 1985). In vitro studies done so far on rattan are of preliminary investigation of a few species. In the near future, in vitro culture should be expanded and perfected involving in a larger

numbers of used in the experimental work on Calamus merillii and Calamus ornatus var. Philippinensis (Umali-garcia, 1985) ; But in Malaysia and Indonesia they emphasized mainly on Calamus manan (Aziah,1985; Gunawan, 1986).

In this project we propose to work with three species that have commercial potential, good quality and widely used in Thailand. Wai Nam Pueng (Calamus sp.) an unidentified species, Wai Takra Thong (Calamus caesius Blume.) and Wai Hom (Calamus pandanosmus Furtado.) which are the most important species that are in short of supply. Wai Nam Pueng (Calamus sp.) has the thick cane for use in making furniture frames with an even and smooth surface. It has equal length between knot (nodes) which is convenient to process. This species has solitary stem but if there is any damage caused to the apex, it will no only promote the production of suckers but also resulted in death, to the primary plant. Thus harvesting of this species of plant can be made once only. Wai Takra Thong (C.caesius) The best cane of the medium size diameter, has the best quality for all types of binding and weaving in furniture industry and is widely used locally in the finest basket ware. Wai Hom (C.pandanosmus) is extremely rare and currently found in only one province and facing extinction. This species, is highly desirable for The Foundation for the Promotion of Spplementary Occupation and Related Techniques (SUPPORT) under the Royal Patronage of Her Majesty the Queen, Large quantities are required for this canes to make rattan wares as folk art, craft and rattan furnitures. This rattan species has a great potential for future expansion under the Royal enterprise for the support of poor farmers to earn extra income during the off-farm seasons. Thus, the three species of rattan that has been selected for study in this project represent the three sizes of canes which are large, medium and small size respectively. Of course, special emphasis will be given to C. pandanosmus which is currently used to produce the high quality products and command higher price in the market. Above all conservation of these spices is the important work and need to be consisdered as an urgent matter before extinction of the species. In recent year, plantation of oil palm and rubber tree has increased considerably in the south and more areas of forest have been increased for replantation of rubber trees. If this trend is continued, many wild species, including C.pandanosmus will not be able to survive in the near future.

Innovative aspects :

Rattan is one of the least-protected groups of the flowering plant. The exploitation of wild rattans are increasing with the global increase in demand for cane furniture. It has been harvested from the forest year after year without any conservation and replantation. In addition, cultivation of rattan has been practical only in limited areas, eventhough it has a great demand for certain species of rattan. The usual method of propagation is by growing rattan from seeds. In the event of difficulties in obtaining seeds, other vegetative propagation methods requiring the use of planting material such as sucker and rhizome, or even stem cutting for some species, would have to be employed. These methods are related to the growth habits and morphology of the species concerned; nevertheless, such methods have been practiced with limited success.

How other organization supported the project.

1. The Royal Development Board supported the budget for the research project before US. Agency for International Development granted to this project for collection some economic species of rattans.

2. Office Trang Province. The Royal Chitralada Projects request the assisting from the govornor to establish Trang Rattan Collec tion Project to collect and planting rattan in Trang province. The enco- ragement for interesting parties in conservation all kinds of rattan pecies by receiving the official co- operation under the leading of Trang's govornor and the district council with the rural villagers in joining together protecting the collection of rattans for to be a source of rattan species of the country and dedicate to H.M. the King Bhumipol. This will result in conservation the various kinds of rattan species and more over to be a source for planting rattan which propagate through tissue culture from The Royal Chitralada Projects and useful for rattan studying and research in the future.

3. Office of District Forestry, Pattanee Province helped this project to collect Wai Takra Thong (Calamus caesius Blume.) from Isatia, Reserving forest.

4. Office of Tung Salanglung National Park helped this project to survey and collect Wai Nam Pueng (Calamus sp.).

5. Narathiwat Rubber Experiment Station, Rubber Research Institute, Department of Agriculture, Ministry of Agriculture and Coopera tive helped this project to plant rattan in the experiment station.

6. Department of Botany, Faculty of Science, Kasetsart Univer- sity helped this project to study Anatomy of rattan apical shoot through tissue culture.

7. Plant Genetic Conservation Project as a Royal initiation of The Crown Princess Maha Chakri Sirindhorn helped the project to study on Rattan identification by Electrophoresis.

METHODS and RESULT

Material and methods

Original of explants

Rattan fruits (Wai Takra Thong Calamus caesius Blume.) from Isatia reserving forest, and seedling from Sukirin, Narathiwat province. For Wai Nam Pueng(Calamus sp.) from Tung Salanglung National Park, seed- and Shoot of Wai Hom (Calamus pandanosmus) had been collected from Pa-toa and Pra-Tew, Chumporn province were employed for the experiments.

Preparation of explants

Surface sterilization of rattan fruits had been made by using 20 % Sodium Hypoclorite for 30 min and immersed in pen-strep antibiotic solution (0.25 gm/100ml sterile distilled water) for 30 min. followed by rinse 3 times with sterile distilled water. Seperated seed coat and fleshy fruit from seed and carefully cut the seed with pruning shears to find the embryo. The embryos had been excised by surgical blade and applied onto the MS medium devoid of plant growth regulators. All culture were incubated at 26 $\pm$ 1°C. for two weeks to obtain clean culture.

Cultural media

The basal medium was Murashige and Skoog (1962), 2,4-D and BAP were contained depend on the experiments and activated charcoal added if necessery. For the semi-solid media 0.2% gelrite was the gelling agent. Nutrient media were dispensed by 1 ml each hole in 24 holes plastic well plate costa and the vessels depended on the experimental work. The pH of media were set at 5.7 prior to additional of gelrite and the media were sterirized by autoclaving 15 min at 15 lbs/ inch^2.

The medium for transplanting were sand and coconut husk dust mixture at ratio 1:1 (V/V) and had been sterilized by autoclaving.

Wai Nam Pueng (Calamus sp.)

Propagation through tissue culture of Wai Nam Pueng (Calamus sp.)

Embryo culture of Wai Nam Pueng (Calamus sp.)

Embryo culture of Wai Nam Pueng (Calamus sp.) on MS medium added with 0, 2 mg/l BAP + 0, 0.5, 1, 2, 4, 8 mg/l 2,4-D in 24 hr./day light condition.

Rresult

Embryo culture of Wai Nam Pueng (Calamus sp.) on MS medium added with 0, 2 mg/l BAP + 0, 0.5, 1, 2, 4, 8 mg/l 2,4-D and o.3% activated charcoal in 24 hr./day light condi-tion appeared that embryo developed to be shoot and root on MS medium added with 0.5 mg/l 2,4-D and specific for shoot formation on MS medium added with 2 mg/l BAP.

Table 1. Embryo culture of Wai Nam Pueng (Calamus sp.) on MS medium with 0.3% activated charcoal and variuos concehtration of 2,4-D and BAP in 24 hr. light condition for 60 days.

2,4-D mg/l	% calus	shoot		root		
		% shoot	height (cm)	% root	amount	lenght (cm)
Control	0	60	3.23	60	1.00	4.50
0.5	0	100	3.13	100	1.25	3.34
1	20	80	1.90	80	1.00	2.38
2	20	80	3.00	80	1.40	4.36
4	33.33	33.33	3.00	33.33	1.50	5.67
8	20	80	2,95	80	1.50	5.08

Callus induction from young leaves of Wai Nam Pueng (Calamus sp.)

Young leaves of Wai Nam Pueng (Calamus sp.) size 0.5x1 cm were applied on MS medium added and without 500 mg/l casein hydrolysate and 2 mg/l BAP + 0, 5, 10, 20, 40, 80 mg/l 2,4-D in dark condition for 60 days.

Result

Callus induction from young leaves of Wai Nam Pueng (Calamus sp.) size o.5x1 cm were applied on MS medium added and without 500 mg/l casein hydrolysate and 2 mg/l BAP + 0, 5, 10, 20, 40, 80 mg/l 2,4-D in dark condition for 60 days obtained callus onevery treatments. Callus produced on leaves or wound with round shape. The callus were yellowish-white . On the medium added with 20 mg/l 2,4-D was the best callus.

Table 2. Cullus induction of young leaves of Wai Nam Pueng (Calamus sp. on MS medium added and without 500 mg/l casein hydrolysate with various concentration of 2,4-D and 2 mg/l BAP in dark condition for 60 days.

2,4-D mg/l	without 500 mg/l casein hydrolysate			500 mg/l casein hydrolysate		
	% callus	% die	Browning	% callus	% die	Browning
Control	0	19.29	+	0	32.14	+
5	2.19	16.06	+	5.36	17.86	+
10	3.17	16.67	+	7.41	18.52	+
20	6.67	15.55	+	18.03	13.11	+
40	3.01	21.18	+	5.66	28.30	+
80	1.74	84,35	+	2.13	85.11	+

Callus induction from young petiole of Wai Nam Pueng (Calamus sp.)

Callus induction from young petiole of Wai Nam Pueng (Calamus sp.) on MS medium added with 500 mg/l casein hydrolysate and 2, 4 mg/l BAP + various concentration of 2,4-D in dark condition for 60 days.

Result

Callus induction from young petiole of Wai Nam Pueng (Calamus sp.) on MS medium added with 500 mg/l casein hydrolysate and 2, 4 mg/l BAP + various concentration of 2,4-D in dark condition for 60 days . Its obtained callus on the medium added with 2 mg/l BAP + 2 - 10 mg/l 2,4-D and MS medium with 4 mg/l BAP + 8-10 mg/l 2,4-D. (Table 3). MS medium added with 2 mg/l BAP and 6 mg/l 2,4-D obtained the best callus for 33.86%. Its produced callus 30 days after cultured. MS medium added with 2 mg/l BAP and 2 mg/l2,4-D obtained less callus for 33.13%.

Table 3. Cullus induction of young petiole of Wai Nam Pueng (Calamus sp. on MS medium added and without 500 mg/l casein hydrolysate with various concentration of 2,4-D and 2-4 mg/l BAP in dark condition for 60 days

2,4-D mg/l	2 mg/l BAP			4 mg/l BAP		e
	% callus	% die	Browning	% callus	% die	Browning
Control	0	0	+	0	0	+
0.5	0	0	+ +	0	10	++
1	0	0	+	0	0	+++
2	33.13	0	+	0	0	+
4	25.00	0	+++	0	0	+++
6	33.86	0	+	0	0	+
8	10.00	0	+	20.4	0	+++
10	25.00	0	++	25.0	0	+++

Callus induction from parts of petiole of Wai Nam Pueng (Calamus sp.)

Callus induction from various parts of young petiole of Wai Nam Pueng (Calamus sp.) such as, (1) leaf sheath near the nodes,(2)leaf sheath, and (3)remain parts on MS medium added with 500 mg/l casein hydrolysate and 2 mg/l BAP + 6 mg/l 2,4-D in dark condition for 60 days.

Result

Callus induction from various parts of young petiole of Wai Nam Pueng (Calamus sp.) such as, leaf sheath near the nodes(1), leaf sheath (2), and remain parts (3) on MS medium added with 500 mg/l casein hydrolysate and 2 mg/l BAP + 6 mg/l 2,4-D in dark condition for 60 days. Its appeared that the tissue near the node obtained the best cullus induction for 75%. Leaf sheath and petiole were obtained callus respectively. (Table 4). After culture 120 days the tissue near the node obtained callus 92.06 %. And appered that from young petiole near the node and leaf sheath obtained direct embryoids.

Table 4. Cullus induction from parts of young petiole of Wai Nam Pueng (Calamus sp.) on MS medium added with 500 mg/l casein hydroly sate with 6 mg/l 2,4-D and 2 mg/l BAP in dark condition for 60 days and 120 days.

parts	Tissue	2 mg/l BAP			4 mg/l BAP		e
		% callus	% Embryoids	% die	% callus	% Embryoids	% die
1	64	75	0	0	92.06	4.76	0
2	230	30	2.61	0	40.42	6.96	0
3	246	11.38	0	0.4	11.38	0	0

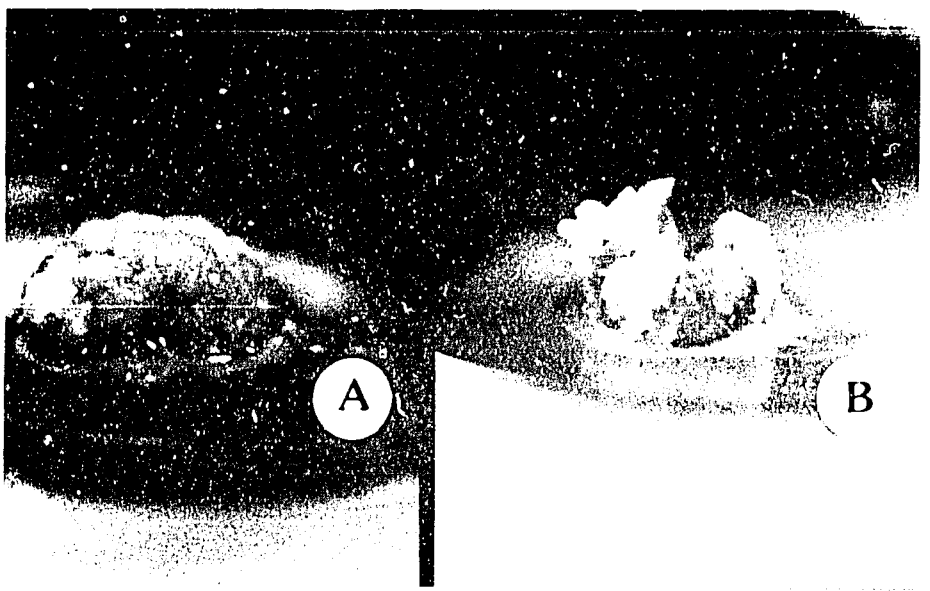


Fig 1. Callus and embryoids from parts of young petiole of Wai Nam Pueng (Calamus sp.) on MS medium added with 500 mg/l casein hydroly sate with 6 mg/l 2,4-D and 2 mg/l BAP in dark condition for 60 days and 120 days.
A. Callus after cultured 60 days
B. Callus afetr cultured 120 day

Embryoids formation from embryo culture of Wai Nam Pueng (Calamus sp.)

Embryoids formation from embryo culture of Calamus sp. were induce cullus cultured on the MS medium added with 2 mg/l BAP + 0.5 mg/l 2,4-D at 0, 12, 24 hr. light conditions for 60 days.

Results

Callus from embryo culture of Calamus sp. were developed to be embryoids in every light conditions after cultured for 30-45 days. The amount of embryoids were not so much different but the type were different. In the dark condition the embryoids were white or pale yellow small cluster. In 12 hrs. light/days the embryoids were yellow and bigger than in the dark and some of them there were green shoot and cultured on the same medium in the dark condition its obtained increasing embryoids for 8 -10 times in 30 days and embryoids developed to be shoots for 90-120 days

Table 5. Embyroids formation from embryo culture of Wai Nam Pueng (Calamus sp.on MS medium added with 0.5 mg/l 2,4-D and 2 mg/l BAP in 0, 12, 24 hr. light condition for 60 days

Light	Embryoids formation	amount of embryoids	characters of embryoids
0	70	45-50	white-cream embryoids, size 0.1x0.1 mm.
12	70	25-30	yellow some were green bigger than in the dark.
24	60	15-18	green big embryoids and differntiated tobe shoots

Embryoids formation from young petiole of Wai Nam pheung (Calamus sp.)

The explant using callus from young petiole cut to 0.8x0.8 cm. and culture on MS medium added with 2 mg/l BAP and 0.1, 0.02, 0.04, 0.06 0.08 and 0.10 mg/l 2,4-D in the dark condition for 30 and 60 days.

Result

Culturing of young petiole on MS medium added with 2 mg/l BAP and various concentration of 2,4-D in the dark condition. Its showed that callus of young petiole developed to be embryoids on every concentration of 0.0.-0.10 mg/l 2,4-D. Callus began to change to be embryoids for single white spots or big cluster and the white spots were developed to be embryoids.

Table 6. Embyroids formation from young petiole of Wai Nam Pueng (Calamus sp.on MS medium added with variuos concentration of 2,4-D and 2 mg/l BAP in dark conditions for 30 and 60 days.

2,4-D mg/l	30 days		60 days	
	Embryoids formation (%)	amount of embryoids per peice	Embryoids formation (%)	amount of embryoids per peice
0.01	20	3.5	40	3.5
0.02	40	11.5	60	11.5
0.04	20	7.5	40	7.5
0.06	20	7.5	40	7.5
0.08	20	4.0	60	4.5
1.0	20	14.0	20	14.0

Table 7. Embyroids multiplication from young petiole culture of Wai Nam Pueng (Calamus sp.on MS medium added with variuos concentration of 2,4-D and 2 mg/l BAP in dark conditions for 30 days.

2,4-D mg/l	amount of embryoids per peice	increasing of embryoids (times)
0.01	25	5
0.02	14.4	2.88
0.04	16.4	3.28
0.06	9.2	1.84
0.08	9.8	1.96
1.0	7.75	1.55

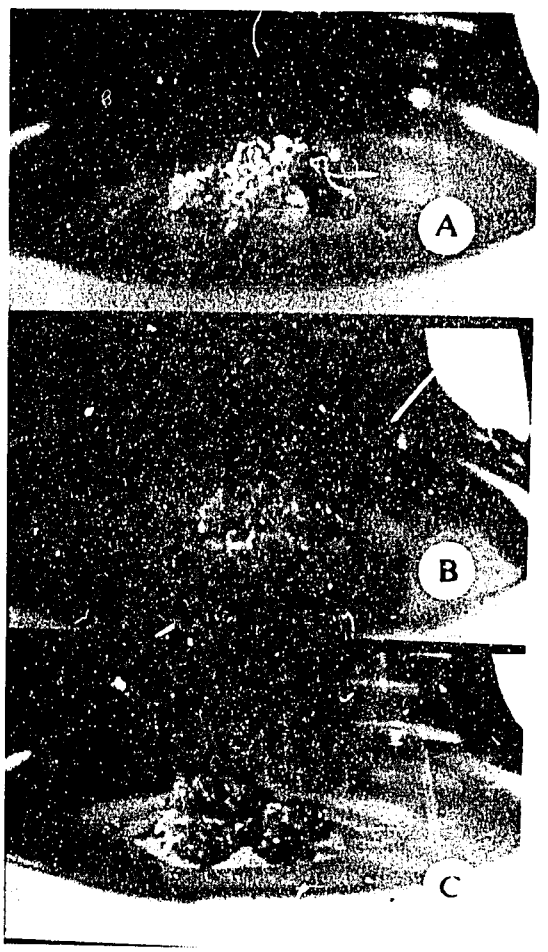


Fig.2 Embryoids of Wai Nam Pueng (Calamus sp.) from cullus culture on MS medium added with 2 mg/l BAP in different light condition for 60 days.

A. Embryoids in dark condition
b. Embryoids in 12 hrs. light/das
c. Embryoids in 24 hrs. light/day

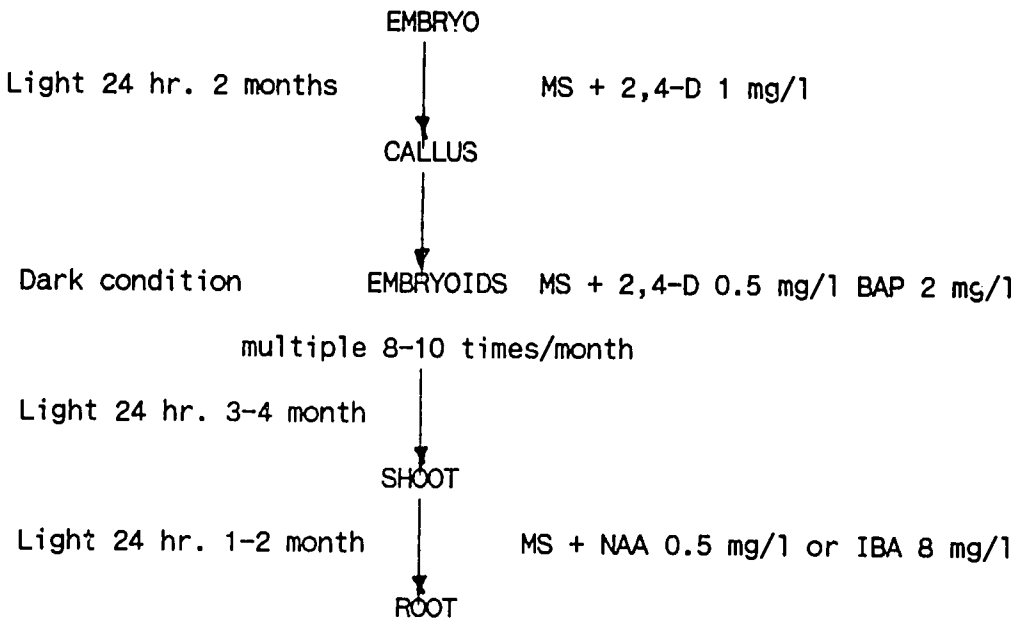


Fig 3. Scheme of Embryo culture Wai Nam Pueng (Calamus sp.)

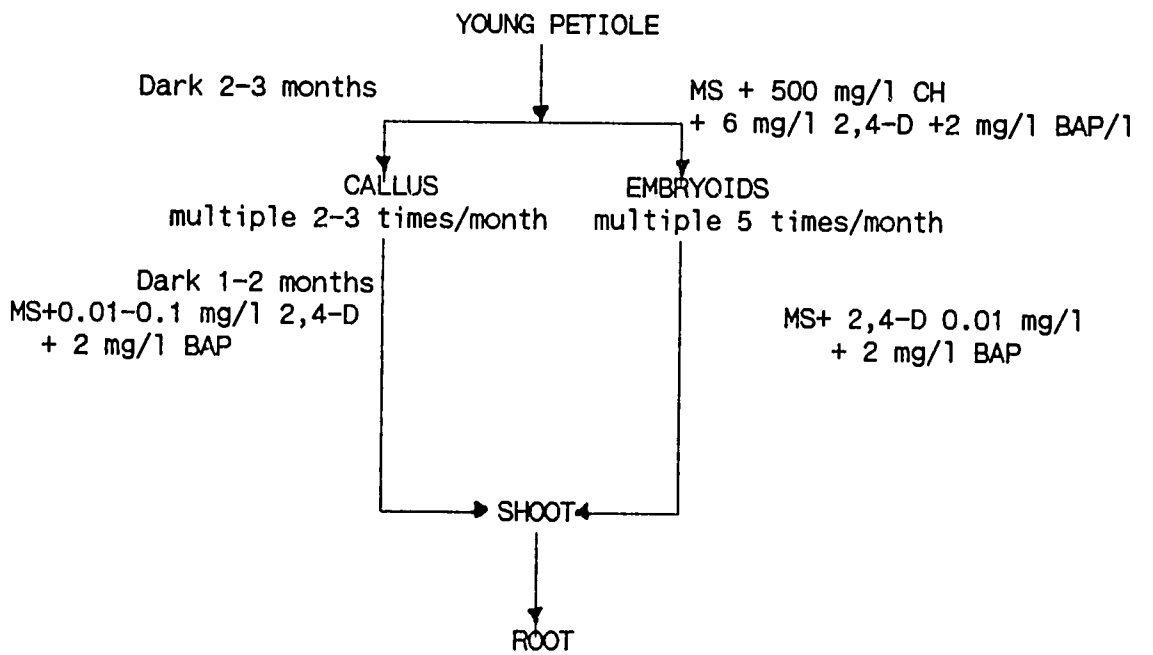


Fig. 4 Organ culture (Young petiole) of Wai Nam Pueng (Calamus sp.)

Tissue Culture of Young Inflorescence of Wai Nam Pueng (Calamus sp.)

Shoot tip of Calamus sp. with young inflorescence were peeled leaf sheath which had 4-5 leaf sheath wraped. Surface sterilization had been made by using 1 % Sodium hypochlorite (NaOCl) and 2-3 drops of Teepol for 30 min. followed by ribsed 3 times with streile distilled water. Excised brack cover the cane and saw the position of young inflorescence between stem and leaf sheath. Young inflorescence were small respectively near apical meristem. There were very small infloresence. Saperated the 3-4 protective spathes and taking young infloresence especially only axil which cylinder acute tip and cut for 8-10 mm. then applied on to the MS medium supplemented with 500 mg casein-hydrolysate 6 mg/l 2,4-D and 2 mg/l BAP. The cultures were incubated under dark condition for 90 days. Obtaining of callus formation and embryoids were recorded.

Results

Culture of young inflorescence of Calamus sp. on MS medium added with 500 mg/l casein hydrolsate + 6 mg/l 2,4-D + 2 mg/l BAP for 90 days appeared that there were 38.8% callus formation. Embryoids and Calli were round shape and size were 2-3 mm. compacted light yellow and dark yellow. Callus formation were obtained after culturing for one month. Young flowers were obtained 31.25 % young flowers and cluster for 5-50 flowers for each piece. The young flowers were white color on different size. The size of flowers were 15 x 2 x 4 mm and tissue which had flowers were size 1 x 1 mm. There were embryoids 16.67 % the shape of embryoids long and acute. Embryoids produced at the wound or scattered. There were 3-15 embryoids for each piece and white color.

Table 8. Culturing of young inflorescence of Calamus sp. on MS medium added with 500 mg/l casein hydrolysate + 6 mg/l 2,4-D + 2 mg/l BAP for 90 days.

Tissue	young folwer formation			Embryoids			Callus formation							
	% formation/young flower/browning per piece			% formation/embryoids/browning per piece			% formation/some of callus/browning/ per piece							% died
								1	2	3	4	5		
144	31.25	5-50	+++	16.67	3-15	++	38.8	0	9	14	17	16	+	13.20

level callus formation
- 1 = non Callus
- 2 = slightly formed callus
- 3 = formed callus more than 2
- 4 = formed callus more than 3
- 5 = formed callus more than 4

Level of Browning
- - no browning
- + less browning
- ++ morderate browning
- +++ more browning

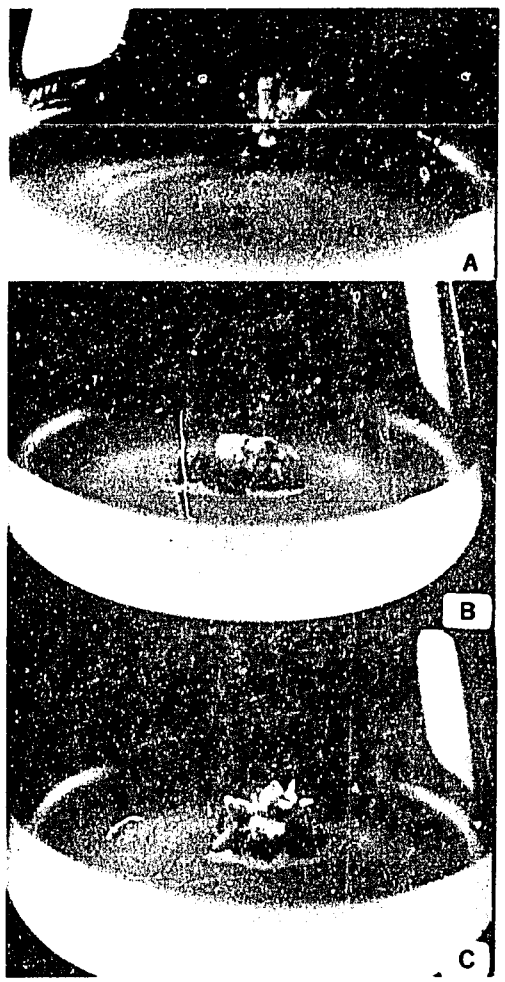


Fig. 5 Differentiation of young inflcresence which cultured on MS medium supplemented with 500 mg/l casein hydrolysate + 6 mg/l 2,4-D + 2 mg/l BAP under dark condition for 90 days.
A. developrd to be young flower
B. developed to be callus
C. developed to be embryo

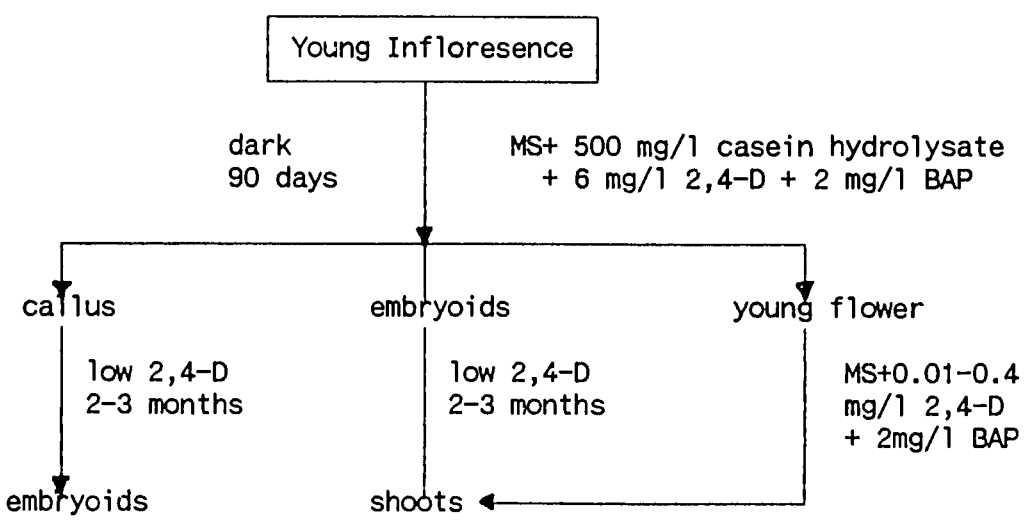


Fig 6. Scheme of Calamus sp young infloresences tissue culture

Culturing of Wai Nam Pueng (Calamus sp.) young flowers.

Young flowers from the previous experiments were cut for cluster. Each cluster tnere were 5-6 young flowers and cultured on MS medium added with 0, 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4 mg/l 2,4-D + 2 mg/l BAP under dark conditions. Growth were recorded after 30- 60 days.

Results

Culturing of Calamus sp. young flowers on MS medium added with 0-0.4 mg/l 2,4-D + 2 mg/l BAP showed that the culturing on MS medium devoid of plant growth regulators could not increase young flower or developed to be shoot. The flowers were browning yellow and same died. Young flower were increased 3.4 times on MS contained with 0.2 mg/l 2,4-D + 2 mg/l BAP (table 9). It showed that 2,4-D and BAP necessiity for increasing and develop to be shoots. The increasing of young flowers were less would be effected from unsuitable medium on the cells which differen tiated to be flowers were limited and shoot formation were less would developed on experiment times.

New young flower were pale yellwow or white the old flower were green and enlarged. The young flower were developed to be shoots on MS medium + 0.01, 0.02, 0.08, 0.2, 0.4 2,4-D after cultured for 2 months. But there were few shoots. The shoots were green and different from shoots which developed from embryoids. The shoots from young flowers at the base there were rachis long 5-20 mm. Sometimes there were growth of shoots on each infloresence and there was one shoots at the end of rachis plantlets were obtained on the rachis. (Fig 8)

Table 9. Culturing of Calamus sp. Young flower on MS medium added with 0.01-0.4 mg/l 2,4-D + 2 mg/l BAP under dark condition for 30 and 60 days.

Tr	2,4-D	BAP	30 days		60 days	
	mg/l	mg/l	increasing of flowers(times)	Shoot formation	increasing of flowers(times)	Shoot formation
1	0	2	1.0	-	1.0	-
2	0.01	2	2.0	-	2.0	5.0
3	0.02	2	1.8	1.0	2.2	1.0
4	0.04	2	2.0	-	3.0	-
5	0.06	2	2.0	-	3.0	-
6	0.08	2	2.2	-	3.0	4.0
7	0.10	2	2.0	-	2.4	-
8	0.20	2	2.4	3.0	3.4	3.0
9	0.40	2	2.4	5.0	3.0	5.0

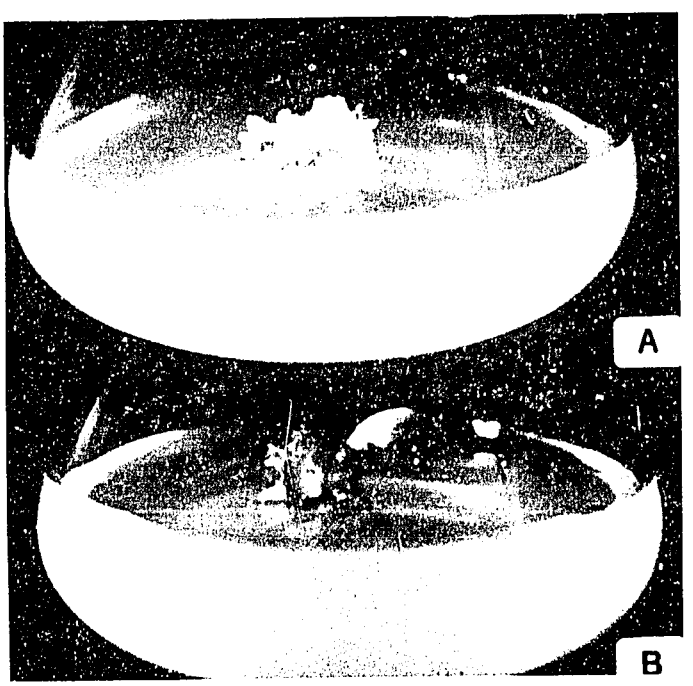


Fig 7. Culturing of Calamus sp. Young flower on MS medium added with 0.01-0.4 mg/l 2,4-D + 2 mg/l BAP under dark condition for 30 and 60 days.

Root induction of Wai Nam Pueng (Calamus sp.)

Root induction of Wai Nam Pueng (Calamus sp.) were induced on MS medium added with variuos concentration 0, 0.25, 0.50, 1, 2, 4, 8, 16 mg/l NAA and IBA in 24 hr.light condition for 30 and 60 days.

Results

Root induction of Wai Nam Pueng (Calamus sp.) were induced on MS medium added with variuos concentration 0-16 mg/l NAA and IBA. Its showed that both of auxins were effected to promote root formation of Calamus sp.. Root formation were depended on various concentration. MS medium + 8 mg/l IBA obtained root formation for 90 % after induced for 60 days . The MS medium + 0.5 mg/l NAA obtained the longest lenght of roots for 4.25 cm. and induce rapid root formation for 15 days.

Table 10 Root formation of Wai Nam Pueng (Calamus sp.) on MS medium added with variuos concentration of NAA and IBA for 30 and 60 days.

mg/l auxin		30 days			60 days		
		Root formation (%)	amount of root	lenght (cm)	Root formation (%)	amount of root	lenght (cm)
Control		0	0	0	0	0	0
NAA	0.25	30	0.5	0.52	50	1.7	2.49
	0.50	70	1.1	1.53	90	2.5	4.52
	1	30	0.8	0.29	70	3.1	2.7
	2	20	0.7	0.17	80	3.1	2.64
	4	0	0	0.	30	1.1	0.70
	8	10	0.1	0.05	30	1.5	0.29
	16	0	0	0	40	1.8	0.29
IBA	0.25	0	0	0	10	0.2	0.15
	0.50	0	0	0	40	0.4	1.13
	1	30	0.3	0.82	50	0.6	2.43
	2	30	0.3	0.34	80	1.6	3.29
	4	50	0.8	0.57	70	1.6	2.41
	8	10	0.1	0.03	90	6.1	2.10
	16	10	0.1	0.08	50	1.6	0.86

AUXIN	mg/l

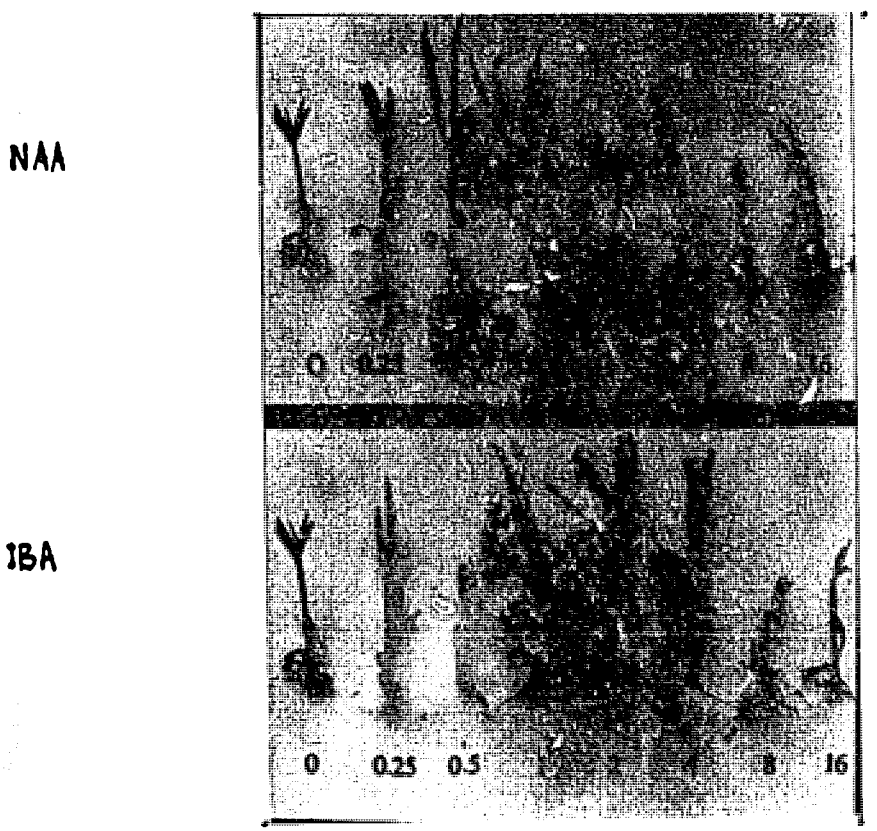


Fig 8. Root formation of Wai Nam Peung (Calamus sp.) on MS medium added with various concentration of NAA and IBA for 60 days.

Wai Takra Thong (Calamus caesius Blume.)

Development of tissue culture techniques for mass propagation

Callus induction of Calamus caesius Blume.

Culturing embryogenic tissue in platic plate (24 well plate Costar) by preparing the MS (Murashige and Skoog 1962),Y3 (Eeuwens 1976) and WPM (Lloyd and McCown 1981)(2,3.4) media with various concentration of 0-10 mg/l 2,4-D and 0-6 mg/l BAP. Finding the appropiate media for callus induction. For comparison between the result of callus induction on MS media contained with variuos combinations and concentrations of auxin (2,4-D;NAA;IBA and picloram 0-10 mg/l) and cytokinin (BAP;2iP;and kinetin 0-6 mg/l). Cutting embryo from clean culture in longitudinal section to 4 pieces and put one piece onto the medium in each hole. Preparing cultures for 2 sets which incubated in the dark 24 hr-daily and in the light 16 hr-daily at 26 $\pm$1°C. Subculturing the explants were done at 14,21 and 30 days.

Results

Comparison for callus induction of MS, Y3 and WPM media showed that on MS medium explants could delevelop to be good callus formation and the growth was better than Y3 and WPM media (Table 11).

Table 11. Callus induction of Wai Takra-Thong (Calamus caesius)on MS Y3 and WPM media in various concentrations of 2,4-D and BAP for 30 days under dark and light conditions.

Media	2,4-D mg/l	BAP mg/l	condition	average	results
MS	0-10	0-6	dark	2.50	produce good creamy callus
			light	2.38	produce white good callus
Y3	0-10	0-6	dark	2.00	produce good callus
			light	1.96	produce callus
WPM	0-10	0-6	dark	1.79	produce callus
			light	1.58	produce callus

Callus induction on MS medium additional with variuos combinations and concentration of auxin and cytokinin such as 2,4-D, IBA, NAA, picloram, BAP, 2iP and kinetin. The results showed that in every combination could induce callus but the selection for the combinations and concentrations were 2,4-D+ BAP and IBA + BAP (Table 12).

Table 12 Callus induction of Wai Takra Thong (Calamus caesius) on MS medium with various combination and concentration of auxin and cytokinin for 45 days under dark and light conditions.

Medium	auxin mg/l	cytokinin mg/l	conditions	Results
MS	0-10 2,4-D	0-6 2iP	dark	less callus formation
			light	less callus formation
MS	0-10 2,4-D	0-6 kinetin	dark	less caiius formation, mostly turn brown and die
			light	less callus formation, mostly turn brown and die
MS	0-10 NAA	0-6 BAP	dark	produce pale yellow callus some form shoot
			light	produce yellowish callus some form shoot
MS	0-10 NAA	0-6 2iP	dark	produce uniform and pale yellow callus
			light	produce uniform and pale yellow pale callus
MS	0-10 NAA	0-6 Kinetin	dark	produce callus
			light	produce callus
MS	0-10 IBA	0-6 BAP	dark	produce whitish compact and hairy callus, large size
			light	produce good compact green callus formation and large size
MS	0-10 IBA	0-6 2iP	dark	produce white compact and large size callus
			light	produce translucent, compact green callus
MS	0-10 IBA	0-6 Kinetin	dark	produce whitish translucent compact and hairy callus
			light	produce translucent and compact callus
MS	0-10 picloram	0-6 BAP	dark	produce white compact and succulent callus
			light	produce good callus formation
MS	0-10 picloram	0-6 2iP	dark	produce callus
			light	produce callus
MS	0-10 picloram	0-6 Kinetin	dark	produce good callus
			light	produce good callus

Callus induction of Calamus caesius in vitro seedling employed the explant from various parts such as cabbage, stem, root tip, young leaf and petiole were conducted by same procedure using MS medium added with various concentration of 2,4-D and BAP.

Callus induction of Calamus caesius in vitro seedling, employed explants from various parts such as cabbage, stem, root tip, young leaf and petiole appeared that the explant from cabbage obtained organogenesis and developed to be 6-10 shoots/vessel. The explant from stems were not successed as same as root tip culture. The callus induction from young leaf on MS medim added with 0.5 mg/l 2,4-D + 6 mg/l BAP under dark condition produced yellow and succulent callus (Table 13).

Table 13 Callus induction of Wai Takra Thong (Calamus caesius) in vitro seedling from various explants and concentrations of 2,4-D and BAP under dark and light condition for 30 days.

Medium	2,4-D mg/l	BAP mg/l	explants	condition	Results
MS	0-10	0-6	cabbage	dark	low conc. of BAP produce white callus and high conc of BAP produce compact callus
				light	less develop callus, high conc of BAP prduce green white compact callus
MS	0-10	0-6	shoot	dark	less develop and succulent callus
				light	2-4 mg/l produce callus
MS	0-10	0-6	root	dark	0.1 mg/l 2,4-D + 2 mg/l BAP form callus
				light	no change of explant
MS	0-10	0-6	young leaf	dark	Mostly browning and died 0.5 mg/l 2,4-D + 2 mg/l BAP produced yellow and hairy callus
				light	no change of explant
MS	0-10	0-6	petiole	dark	slightly enlarge explant and produce at high conc. of BAP.
				light	Mostly no change of explant and browning

Callus multiplication of Calamus caesius Blume.

The explants were calli of Calamus caesius from previous experiment which had cultured onto the MS medium devoid of plant growth regulator for 2 weeks. Preparing the selected media added with 0.1-0.5 mg/l 2,4-D and 2-4mg/l BAP filled in plastic plate. The calli were cut in size 3x3 mm and cultured onto the media. After second subculturing calli were transfered onto the same media in the vessel. All cultures were incubated under different conditions in the incubating room and growth chambers.

The studying on multiplication of Calamus caesius callus under different conditions appeared that the callus could multiple at 30°C under the dark 24 hrs-daily on MS medium added with 0.5 mg/l 2,4-D + 2 mg/l BAP was the best. (fig.9)

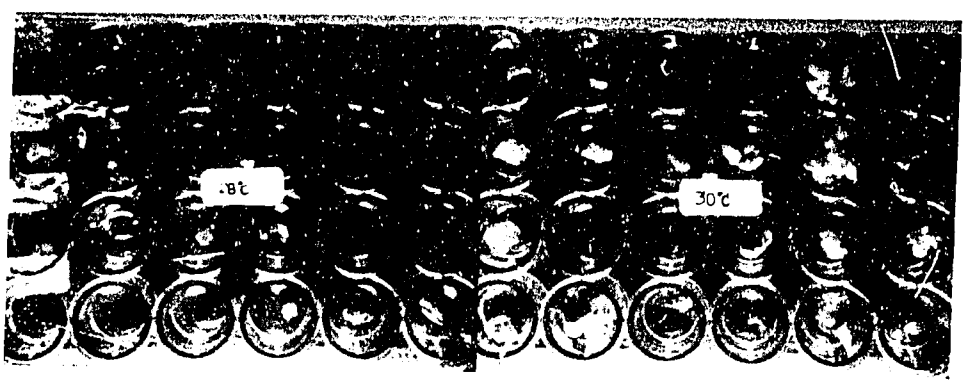


Fig 9. Callus multiplication of Calamus caesius Blume.

Shoot formation

Preparing the medium which selected, MS medium added with 0.1-0.5 mg/l 2,4-D and 2-4 mg/l BAP. The calli were transferred onto the media and incubated under 16 hr-daily light and 24 hr-daily dark conditions.

The development of callus on MS medium with 2,4-D and BAP under 16 hr-daily light condition showed better result on callus enlargement and shoot formation than under the dark. Callus development to be embryo genesis and shoot formation in 45 days.

Shoot multiplication employed 4-5 multiple shoots per piece was was better than using single shoot and multiplication under light condition were better than under the dark condition.

Fig 10. Shoot formation of Calamus caesius Blume.

Root formation

Selection the heightest shoot fron multiple shoots to be single and subcultured onto MS medium devoid of plant growth regulators added with activated charcoal.

Root formation of Calamus caesius were obtained 100 % for one month on MS medium devoid of plant growth regulator. Auxin in previous medium were enough for rooting. For our study the culturing on the medium without activated charcoal appeared browning and inhibit growth of plantlets when compared with culture on the medium added with activated charcoal. Browning which were commonly in palm tissue and absorbed by activated charcoal.

Effect of modified media on rooting of Calamus caesius blume.

The MS medium added with activated charcoal normally induce the rooting of Calamus caesius within 1 month. The period of root induction is probably minimized by various component of the media. This show the reducing cost and time on culturing Calamus caesius. The specific objective is to test the optimum component of the medium for root induction of Calamus caesius.

Materials and methods

The following component of the media were tested :

Tr.1. MS medium added with 0.2 % activated charcoal as control.

Tr.2. 1/2 MS added with 0.2 % activated charcoal.

Tr.3. 1/2 of KNO_3 in MS medium added with 0.2 % activated charcoal.

The explants were seedling with 6-7 cm height and 5-6 leaves were used. Fifteen of explants were cultured in each treatment.

Results

The results showed in the table 15 demonstrated that Calamus caesius cultured on 1/2 MS added with 0.2 % activated charcoal were rooted 100 % and could be transplant in 5th week. The plantlets on 1/2 KNO3 are also transplanted only 46 % . This is in contrast to the control that only 33 % can be transplanted. Therefore the half concentration of MS medium added with 0.2% activated charcoal seem to be a suitable medium for rooting medium of Calamus caesius. The plantlets are waiting for further study.

Table 15. The results of various component of the medium on the height, and root of Calamus caesius Blume.

Treatment	Time weeks	Height cm.	% rooting from total plantlets	% transplant
Tr 1	0	7.20	0	0
Tr 2	0	6.40	0	0
Tr 3	0	6.70	0	0
Tr 1	1	7.70	0	0
Tr 2	1	6.40	0	0
Tr 3	1	6.70	0	0
Tr 1	2	8.90	5.80	0
Tr 2	2	7.60	31.00	0
Tr 3	2	7.50	0	0
Tr 1	3	8.90	24.00	0
Tr 2	3	8.30	60.00	0
Tr 3	3	7.60	41.00	0
Tr 1	4	9.40	52.00	0
Tr 2	4	10.00	100.00	0
Tr 3	4	8.40	46.00	0
Tr 1	5	10.05	59.00	33.33
Tr 2	5	10.41	100.00	100.00
Tr 3	5	9.11	47.00	46.60

Transplanting

Plantlets from embryo culture amd organogenesis were rinsed in distilled water to remove residual nutrient medium and soaked with fungicide before planted to plastic pot contained 1 sand: 1 coconut husk dusk mixture (v/v). Transplant medium were sterilized by autoclaving. The plantlets were placed in glass chamber 12x24x16 inchs. and wraped top of chamber by clearing plastic film. After 2 weeks, plantlets in plastic pots from glass chamber were transplanted in 5 inchs plastic bags and placed in the nursery with 70 % shade.

Results

Transplanting in the nursery were successful. The plantlets from embryo culture which transplanted in the nursery were survived for 84.6 % and the plantlets obtained from organogenesis after transplanted were survived for 90.4 %(Table12). The 2 months old plantlets from organogenesis would be more vigor than plantlets from embryo culture which were 8 months old. The problem after transplant into plastic bags were drying and some ofthem were stem rotted at the base and root. Plantlets supplied with sufficient nutrients in a rigidly controlled environment free contamination, would have undergone a shock when transferred to untreated medium. Other facters that could have increased susceptibility to fungal infection.

Table 15 Transplanting of Calamus caesius plantlets from embryo culture and Organogenesis.

Culture	Total (plants)	survived plantlets (plants)	percent
Plantlets from Embryo culture	312	264	84.6
Plantlets from Organogenesis	165	149	90.4

Clonal selection of Wai Takra-Thong Calamus caesius Blume. Through Tissue Culture

In vitro seedlings from embryo culture of Calamus caesius Blume. were variation on growth. The objective of this experiment is to select clones which have good quality on growth, rooting and vigor for transplant.

Materials and methods

In vitro seedling of Calamus caesius Blume. were induced shoots from cabbage (portion at the base between shoot and root). The explants were cultured on MS medium contianed with 20 mg/l BAP under light condition 16 hrs/day at 25°C for 1-2 months for shoot induction. Subculturing the explants on MS medium contained with 0.5 mg/l 2,4-D and 2 mg/l BAP for shoot multiplication. Seperate the shoot which 4-5 cm. height for root induction on MS medium added with 0.2 % activated charcoal. Recording on height, amout of leaves and rooting of the cultures from various clones.

Results

Induction for multiple shoots

Shoot induction of Calamus caesius on MS medium contained with 20 mg/l BAP for 1-2 months obtained 2-3 shoots. If cultured on this medium more than 2 months they were browning, succulent and die because of high concentration of cytokinin. Therefore subculturing on MS medium 0.5 mg/l 2,4-D and 2 mg/l BAP obtained multipleshoots. They could subculture for 6-8 times and then must be induce the base of shoot again because of less totipotency.

Root induction

Normally Calamus caesius was easy to obtain root on shoot induction medium and might be seperated the highest shoot from them, it cause to inhibit growth, height and reduce vigorous of the culture. Shoot would induce root on MS medium added with 0.2% activated charcoal. The rooting capability of various clones were difference. From this experiments were selected for 32 numbers for early transplanting which show as follow

2nd week were No.162,169
3rd week were No. 11,20,26,57,82,130,136
4th week were No. 10,65,68,73,86,87,98,112,122,166
5th week were No. 17,33,52,64,67,77,81,85
6th week were No. 18,38,69,119,117

For observation showed that Calamus caesius were transplanted in 4 weeks by average. Growth and vigorous of seedling are further study.

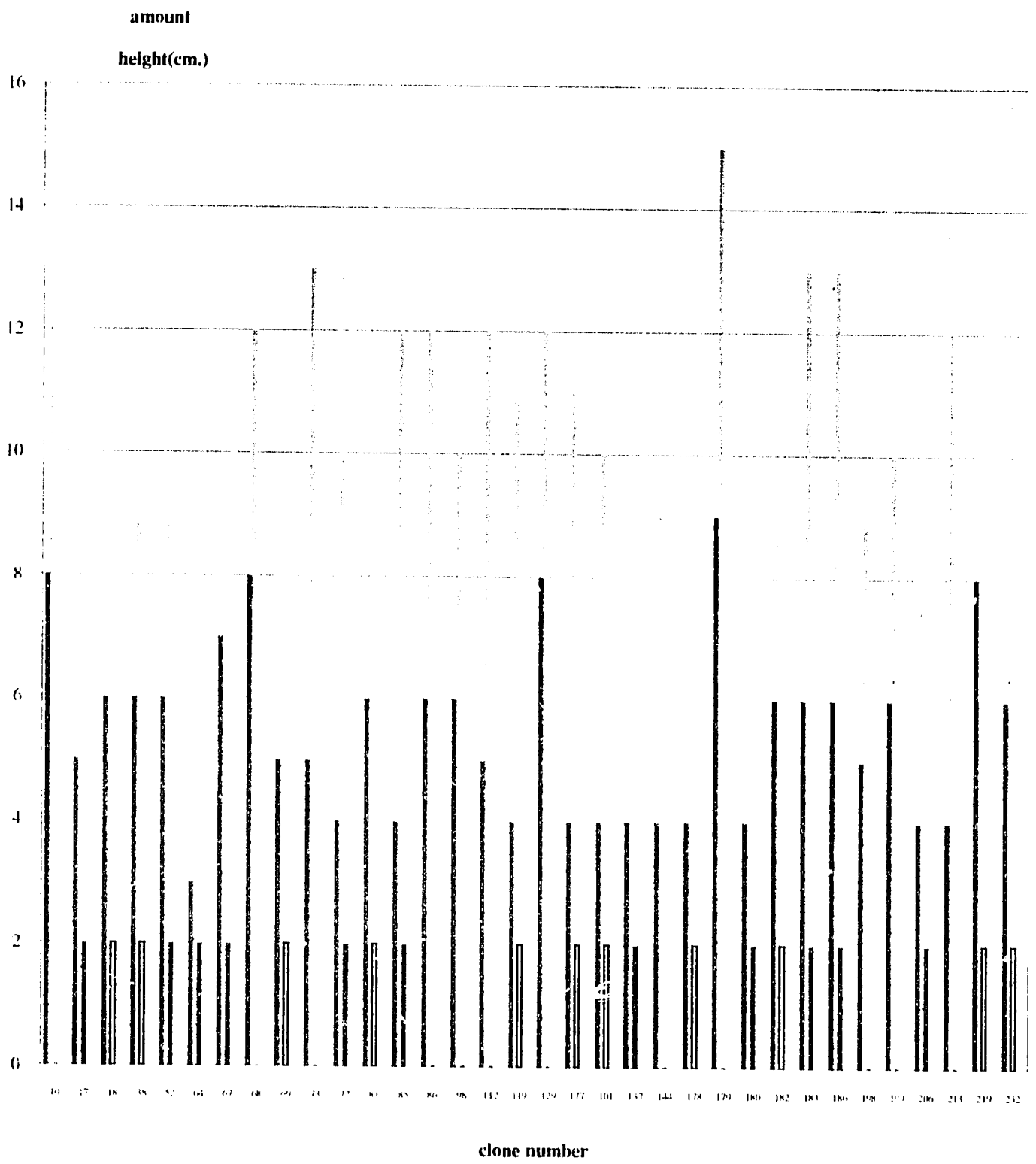
The capability of rooting could induce primary root and waiting for obtained secondary root and could transplant.

Observation on growth there were incresing of height from 4.5 cm. of shoots which induction of root for transplants appeared that :

No increasing of height was No. 130
1 cm. increasing of height were 33, 169
2 cm. increasing of height were 11, 65, 82
3 cm. increasing of height were 64, 68, 69, 112, 122, 136, 166
4 cm. increasing of height were 18, 20, 38, 52, 26, 86, 87, 177
5 cm. increasing of height were 17, 81, 98, 119
6 cm. increasing of height were 10, 57, 67, 73, 77, 85, 165

It showed that the various of clones were difference on growth could induce root and transplant. From the data could select clones which had good characteristic for mass propagation.

Fig 11. Comparison on growth (amount of leaves, height of stem, root formation of various clones of Calamus caesius Blume.



Collection and regeneration of Wai Takra Thong (Calamus caesius Blume.)

Materials and method

Collection seed of Calamus caesius and cultured embryo to induce in vitro seedling. The seeling from embryo culture were cut and use cabbage or base of stem for explant. Culture the explants on MS medium added with 20 mg/l BAP for 2-3 months and select the culture which had 2-3 shoot for subculture. The meduim for subculture was MS added with 0.5 mg/l 2,4-D and 2 mg/l BAP and subculture for every month. Seperated the biggest shoot for root induction on MS medium added with 0.2% activated charcoal.

Results

From induction the explants from base of stem of Calamus caesius on MS medium added with 20 mg/l BAP for 3 months appeared that that they were enlarged and obtained multiple shoots. There were vitrification for cultured on this medium. Subculture on MS medium contanined with 0.5 mg/l 2,4-D and 2 mg/l BAP for 2 weekks there were increasing of multiple shoots. Seperated the biggest shoot for root induction on MS medium added with 0.2 % activated charcoal obtained root in one month. For Calamus caesius were cultured on MS medium add with 2 mg/l BAP and could multiple for every 2-4 weeks.

To date there are 200 clones collect at Plant Tissue Culture Laboratory. the Royal Chitralada Palace.

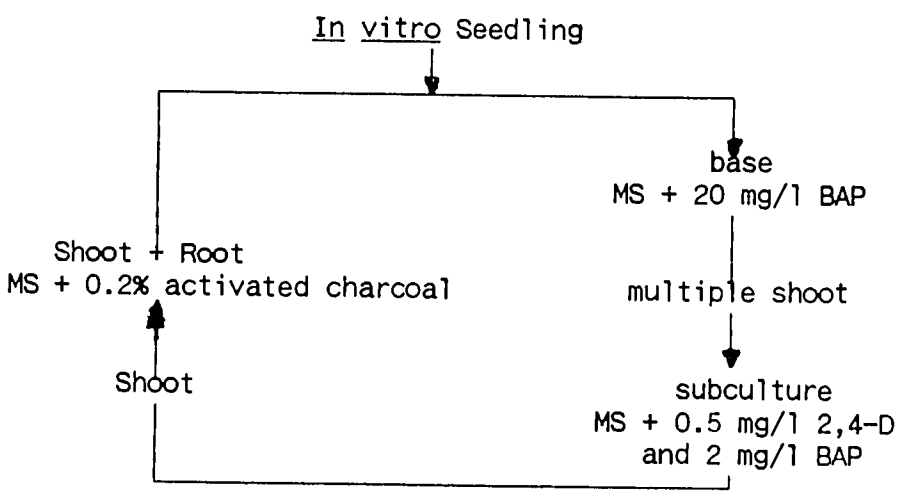


Fig. 12 Scheme of regeration new plant from in vitro seedling of Calamus caesius Blume.

Wai Hom (Calamus pandanosmus) Furtado.

Propagation through tissue culture of Wai Hom (Calamus pandanosmus) Furtado.

Callus induction of Wai Hom (Calamus pandanosmus Furtado.)

Material and methods
Origin of explants

Rattan fruits of Calamus pandanosmus Furtado. from Chumporn province were collected and use for explants.

Surface strilization of Wai Hom (Calamus pandanosmus) fruits had been made by using 20% Sodium hypoclarite (Na OCl) and 2-3 drops of Tween20 for 1 hour followed by rinsing 3 time with sterile distilled water. Exised the embryos from the seeds and applied on to the MS medium added with 0-12 mg/l 2,4-D , 0-12 mg/l 2iP, 0-2 mg/l Picloram. Combination of 0-10 mg/l 2,4-D and 0-6 mg/l BAP. And combination of 0-1.6 mg/l NAA and 0-1.0 mg/l kinetin.

All experiments had been studied in dark and light condition for 6 week. Growth and development of the embryos were recorded every week and callus formotion were recorded in 5 levels as follow 0 = died, 1 = non callus, 2 = Slightly formed callus, 3 = formed callus more than 2 and 4 = formed callus more than 3.

Results

Embryo culture of Calamus pandanosmus embryo s on MS medium added with 0-12 mg/l 2ip. Callus induction in this experiment in dark condition obtained slightly callus and in light condition obtained poor callus and most of treatments usually died and mormally every treatments are not good. The best concentration of 2ip for obtaing the best callus was 10 mg/l in dark condition.

Callus induction in this experiment in dark and light condition could be compared because the result were happened different. In the dark condition, most of concentration obtained calli and some shoot and light condition obtained shoots, but normally every treatments were not not good. The best conncentration of 2,4-D for obtaining the best callus is 8 mg/l 2,4-D in the dark condition.

On MS medium added with 0-10 mg/l picloram for 6 weeks appeard that MS medium + picloram 1.5 mg/l in dark condition produced callus and had average level of callus formation = 1.05. The calli were round shape and slight succulent. Callus formation in dark condition were better than light condition. Callus on MS medium with picloram were white-yellow and compact but in light condition the callus were succulent. On the medium added with 0.5 mg/l 2,4-D + 2 mg/l BAP obtained white and compact callus in the dark condition and yellow - green compact in the light condition. Some shoots were found on some parts of these calli.

On MS medium added with 0-10 mg/l 2,4-D and 0-6 mg/l BAP for 6 weeks appeared that callus formation were very poor through there were no growth rate and callus formation. The average level of callus formation were not increased during the time of experiment but kept on decreasing. Some treatment had no average level of callus formation because the embryos turned brown and died.

On MS medium added with 0-1.6 mg/l NAA and 0-0.1 mg/l Kinetin callus induction in this experiment in dark and light condition obtained callus the same results but in light condition, callus was white, green and have same shoot and root. In dark condition, callus is white, yellow and have some root. Especially in 1.6 mg/l NAA obtained more callus. (Table 16)

Table 16 Embryo culture of Calamus pandanosmus Furtado. on MS medium added with various auxin and cytokinin in dark and light conditions for 45 days.

MS medium	Growth			
	dark	level of callus	light	level of callus
0-12 mg/l 2iP	poor callus	1.05	poor callus & died	0.30
0-12 mg/l 2,4-D	shoot	1.4	shoot	0.79
0-2 mg/l Picloram	poor callus	1.14	poor callus	1.80
0-10 mg/l 2,4-D + 0-6 mg/l BAP	poor callus	0.22	poor callus	0.71
0-1 mg/l NAA + 0-1 mg/l kinetin	shoot & root	2.22	shoot & root	2.00

level of callus formation
0 = died
1 = non callus
2 = Slightly formed callus
3 = formed callus more than 2
4 = formed callus more than 3

Fig. 13 In vitro seedling of Calamus pandanosmus Furtado.

Conservation aspects.
Development of tissue culture for *in vitro* conservation.

Effect of temperature on growth of *Calamus caesius* Blume. multiple shoot and seedlings.

This experiment was a on conservation by low temperature, 7-9 multiple shoots / vessel from organogenesis of *Calamus caesius* which cultured on MS medium added with 0.1 mg/l 2,4-D + 4 mg/l BAP and seedling 10 cm. height cultured on MS medium + 0.2 % activated charcoal. All cultures were incubated at 5°, 10°, 20°, and 25° C for two months in growth chambers under dark conditions.

Studying effect of low temperature on rattan multiple shoots, *in vitro* seedling and embryoids. The multiple shoots of *Calamus caesius* incubated at 5°C and 10°C turned brown and die in one week. Seedling could survive and growth very slow at 5°C and 10°C. (Table 17)

Table 17 Effect of temerature on growth of *Calamus caesius* multiple shoot and seedlings

Treatments	multiple shoot increasing shoot	percent	Seedling increasing of height (cm)	percent
Lightness				
25°C control	6.9	100	4.0	100
Darkness				
25°C	6.7	97.1	1.3	32.5
20°C	1.5	21.7	1.0	25.0
10°C	0	0	0	0
5°C	0	0	0	0

REMARKS At 20°C multiple shoots could not survive, calli were brown and died in 2 months
At 5°C and 10°C multiple shoots were brown and died in one week.
2 months seedling could transplant, they had secondary root . Seedling were not healthy in the darkness.

Effect of growth retardant (Daminozide, chlormequat CCC) growth inhibitor (Abscisic acid ABA) and Mixed media on preservation of *Calamus caesius* Blume.

When transplant of *Calamus caesius* is prohibited by cultureed on inappropiate condition like bad wheather, the preservation of Calamus caesius is very important. The preservation must be done under aseptic condition with minimum incubated area. The optimum medium is, therefore, essential as to preserve rattan for future use. The specific objectives of this experiment is to test the effect of growth retardant (daminozide, CCC, ABA) and Mixed media on preservation of *Calamus caesius* Blume.

Materials and methods

In vitro explants as multiple shoots were prepared. The control media for seedling and multiple shoots were MS plus 2 % activated charcoal and MS medium containing with 0.5 mg/l 2,4-D and 2.0 mg/l BAP respectively. The growth retardant and their concentrations were 250,500, 1,000, 1,500 and 2,00 mg/l for daminozide, CCC and 250, 500, 1,000 mg/l

ABA. The media containing with growth retardant for culturing seedling and multiple shoots. All cultures were incubated at dark condition for three months prior to observation of the growth of explants.

Results

The effects of growth retardant and growth inhibitor and Mixed media on growth of seedling and multipleshoots were shown that at concentration lower than 1,000 mg/l of CCC, only 50% of multiple shoot could survive. At concentration greater than 1,00 mg/l the retardant caused yellowish leaves with multiple roots. Daminozide at 1,500 mg/l produced no increase in height of seedlings. ABA at all concentrations caused browning and no multiple shoot of seedling while damage multiple shoots. Mixed media produce small increase in height of seedling and no shooting of multiple shoots . Only the seedling is shown to be suitable for preservation for further transplant. The growth retardant like daminozide is useful only at concentration not greater than 1500 mg/l. The high concentration of daminozide 2000 mg/l is toxic to both of the seedling and the multiple shoots. (Table 18)

Table 18 Effect of growth retardant (Daminozide, chlormequat CCC) growth inhibitor(Abscisic acid ABA) and Mixed media on preservation of Calamus caesius Blume. for 90 days.

Treatments	% height increasing of seeding	% survive of multiple shoots	Remark
Control	100	100	seedling grew up to 12 cm. and multiple shoots grew to 5-6 shoots.
CCC 250 mg/l	57.14	50	seedling grew up to 7.0-8.5 cm. all multiple shoot were died.
500 mg/l	42.80	50	
1,000 mg/l	57.14	50	
1,500 mg/l	57.14	25	
2,000 mg/l	48.80	25	
Daminozide			
250 mg/l	57.14	75	seedlings grew up 8.4-10.0 cm. all multiple were died.
500 mg/l	42.80	75	
1,000 mg/l	57.14	0	
1,500 mg/l	0	0	
2,000 mg/l	0	0	
ABA 250 mg/l	0	0	all of multiple shoots were died and seedlings were brown.
500 mg/l	0	0	
1,000 mg/l	0	0	
Mixed media	28.50	0	seeding grew up to 9.5 cm. all multiple shoots were died.

Effect of low temperature on growth of Calamus sp. embryoids.

Embryoids of Calamus sp. which cultured on MS medium added with 0.5 mg/l 2,4-D + 2 mg/l BAP were employed for the experiment. The cultures were incubated at 5°C, 10°C, 20°C and 25°C for 60 days in growth chamber under dark condition. There were 10 samples for each treatment. The cultures were obserded and recorded for every week.

Results

After one month of incubation in various temperature the growth of embryoids from 25°C (control), 20°C, 10°C, and 5°C were increased 100%, 27.30%, 4.50%, and 3.50% respectively. For the embryoids incubated at 10°C there were new and healthy embryoids but at 5°C they they grew too slow and were not good embryoids and callus.(Table 19)

Table 19 Effect of temperature on growth of Calamus sp. embryoids in dark conditions for 60 days.

Treatments	Embryoids		
	Starting weight (mg)	1 month weight (mg)	percent of increasing weight
Darkness			
25°C	127.0	577.0	100
20°C	161.3	274.1	27.3
10°C	154.3	174.4	4.5
5°C	139.9	155.6	3.5

Effect of growth retardant, growth inhibitor and Mixed media on preservation of Calamus sp.

In vitro explants as embryoids were prepared. The control media seedling and embryoids were MS plus 2% activated and MS medium containing with 0.5 mg/l 2,4-D and 2.0 mg/l BAP respectively. The growth retardant and their concentration were 250, 500, 1,000, 1,500 and 2,000 mg/l for daminozide, CCC and 250, 500, 1,000 mg/l for ABA(abscissic acid). The media containing with growth retardant for culturing seedling and embryoids. All cultures were inculbated at dark condition for there month period to observation of the growth of explants.

Result

The effects of growth retardant and growth inhibitor and Mixed media on growth of seedling and embryoids were shown in Table 21. Only the seeding can be preserve for future transplant. At concentration lower than 1,000 mg/l of CCC, 60-80% of embryoid could survive. At con centration greater than 1,000 mg/l the growth retardant caused yellowish leaves. Daminozide at 1,500 mg/l produced no increasing in height of seedling. ABA at all concentrations caused browning and no growth of seedling while damage embryoids. Mixed media produce very small increase in height of seedling and increase in weight of embryoids.

Table 20 Effect of growth retardant (Daminozide, chlormequat CCC) growth inhibitor(Abscisic acid ABA) and Mixed media on preservation of Calamus sp.. for 90 days.

Treatment	% height increasing of seedling	% survive of embryoids	Remark
Control	100	100	Seedling grow up to 12 cm. and embryoids increased in weight.
CCC 250 mg/l	88.13	80	Seedling grow up to 8 - 9 cm and embryoids were deid
500 mg/l	58.63	60	
1,000 mg/l	44.60	60	
1,500 mg/l	54.68	40	
2,000 mg/l	74.82	40	
Daminozide			
250 mg/l	29.50	90	Seedling grew up to 9-10 cm. and embryoids were died
500 mg/l	10.80	70	
1,000 mg/l	16.55	30	
1,500 mg/i	0	20	
2,000 mg/l	0	0	
ABA 250 mg/l	0	0	All of embryoids were died and seedling were brown
500 mg/l	0	0	
1,000 mg/l	0	0	
Mixed media	1.02	0	Seedling grew up to 5-6 cm. embryoids were died.

Germplasm collection

1. Collection rattan seedling which collected from the natural forest and take care in the nursery of the Royal Chitralada project and Dept. of Forest-Biology Faculty of Forestry, Kasetsart University.

2 Collection by planting in Dipterocarps Demonstration Forest on Chitralada ground. The area of the forest for 2 acers collected and planted rattans 20 species amount 150 plants.

3. Trang Rattan Collection Project. The Royal Chitralada Projects request the assisting from the govornor to establish the rattan collection and plantation in Trang province. The encoragement for interes ting parties in conservation all kinds of rattan species by receiving the official cooperation under the leading of Trang's govornor and the district council with the rural villagers in joining together protecting the collection of rattans for to be a source of rattan species of the country and dedicate to H.M.the King Bhumipol of Thailand. This will result in conservation the various kinds of rattan species and more over to be a source for planting rattan which propagate through tissue culture from The Royal Chitralada Projects and useful for rattan studying and research in the future.

DISCUSSION

Propagation of rattans through tissue culture of this project showed that the medium added with activated charcoal encorage growth of embryo culture of rattan because of activated charcoal could absorbed subtance in the media (Fridborg and Erikson, 1975, Reynolds 1985). Phenolic compound which produced from metabolite were decreased growth of plant tissue and acitvated charcoal encorage embryo culture of palms species (Wang and Huang 1976). Rattans embryo could not develop to be callus would be less of auxin. Paranjothy and Othman (1982) reported that the culturing of oil palm callus would increase concentration of auxin for ten times in the media which added with activated charcoal. Reynolds and Murashige (1979) could induce immature embryo of Date palmlm (Pheonix dectylifera L.) to produce callus on MS medium added with $5x10^{-7}M$ 2,4-D + $5x10^{-6}M$ N^6-r, r - dimethylallyaminopurine and 0.3 % activated charcoal. Tisserat (1979) could induce date palm callus on the medium added with 100 mg/l 2,4-D + 3 mg/l 2iP and 0.3 % activated charcoal. Aziah and Manokaran (1985) reported that embryo culture of Calamus manan Miq. on the high auxin medium could induce embryo to be shoot and root. Embryo culture of Wai Nam pueng (Calamus sp.) on MS medium added with 0.5- 8 mg/l 2,4-D obtained callus. The response of rattan species on various concentration of plant growth regulators depend on differnt of genetic. Gamborg and Skyluk (1981) reported that genetic is a factor which control growth development of tissue and morphogenrsis of plant species. Embryo culture of Wai Nam pueng (Calamus sp.) on MS medium added with 2,4-D, the embryo developed to be callus better than the embryo which cultured on the medium added with BAP. The Embryo of Wai Nam Pueng (Calamus sp.) obtained more callus on MS medium added with 1 mg/l 2,4-D was appropiated for induction because 2,4-D promoted cell differentiation to be callus (Leopold, 1963) as same as the report of Dekker and Roa (1987) which cultured embryos of Calamus trachycoles could induce and multiplication callus on the medium added with 2 mg/l 2,4-D for 8 weeks. Callus induction from young leaves of Wai Nam Pueng (Calamus sp.) and Wai Ta kra Thong (Calamus caesius Blume.) on MS medium added with various concentra tion , auxin and cytokinin which ovtain callus depend on various plant growth regulators. According to BAP and 2,4-D promoted for cell division and cell enlagement (Skoog and Miller 1957) 2,4-D an auxin encorage RNA and nucleic acid synthesis (Leopold and Kriedemann,1975) which plants synthesis protien and obtained callus. Callus formation would be differen tiated from vascular tissue which could be division and formation from meristematic tissue of young leaves. For culturing young leaves of Wai Nam Pueng, casein hydrolysate were effected to obtained callus, the culture on the medium without casein hydrolysate were not produce callus. Its showed that the tissue obtained callus on the MS medium added with casein hydrolysate to increased nitrogen to the tissue (Dougall 1980, Gamborg and Skyluk, 1981). Additional of casein hydrolysate had many reports for callus induction, growth and development of some plants such as oil palms(Nwakwo and Krikorian, 1983; Thomas and Rao, 1985).

The culturing some parts of young petiole of Wai Nam Pueng (Calamus sp.) on MS nedium added with 500 mg/l casein hydrolysate and 6 mg/l 2,4-D for 120 days, appeared that young petiole near the node obtained callus 92.06 percent which had leaf trace or vascular tissue and had meristematic tissue. Evans and Sharp (1981) presented, embryoids could develop directly from the tissue which differentiated from embryoid

cell. This diefferentiation need the appropiate of plant growth regulator and environment. For Wai Takra Thong (Calamus caesius Blume.), callus induction were not good which succulent and could not multiply and died because of the tissue were cultured onto the medium which unsuitable of plant growth regulator. Some of tissue which culture on the medium added with casein hydrolysate obtained shoot as same as a report (Aziah 1987b) ther were shoot formation from petiole of young seedling obtain shoots in 2 - 3 months on the low cytokinin medium. Rabechault et.al (1970) founded that the medium added with 200-1000 mg/l casein hydrolysate help shoot of oil palm grew well.

Callus culture of Wai Nam Pueng developed to be embryoids in dark and light conditions on the MS medium added with 2 mg/l BAP and 0.5 mg/l 2,4-D. In the dark condition embryoids were small, white or pale yellow which had 45-50 embryoids for each piece. In the light condition embryoids were bigger, green and seem to be shoot. In all times light condition obtained 15-18 embryoids per piece. In the dark condition suitable for embryoids induction than in the light. Tissue in the dark condition would have more cell division than in the light which related amount of auxin. In the light auxin would be break down and less of cell division. Embryoids incubated in the light condition were green and seem to be shoot because light would be encorage cholrophyll synthesis and diferrentiate to be shoot. There were reports for embryoids formation in the dark and light condition. For embryoids induction in the light such as oil plam of Martin and Rebechault (1976), Nwankwo and Krikorian (1983) Date palm of Tisserat and Demason (1980), Christmas palm (Veitchia merrilli Bacc.) of Srinivasan et al. (1985), Perl millet (Pennisetum americanum) of Vasil and Vasil (1981), rattan of Aziah and Manokaran (1985), Allium fislulasam L. of Shahin and Kaneko (1986). The culturing of embryoids on MS medium added with 2 mg/l BAP + 0.5 mg/l 2,4-D, could increase embryoids for 8-10 times in 30 days and embryoids developed to be shoots on the same medium. Its showed that this medium was suitable for embryoid finduction, multiplication and shoot formation.

Callus of young petiole developed to be embryoids after cultured for 30 days on MS medium added 2 mg/l BAP + 0.01-0.10 mg/l 2,4-D Evans et al. (1981) presented the embryoids were started from callus induction on the medium added with high auxin. Callus cell developed to be embryoids which cultured on low auxin medium. It as same as culturing rattan which induced callus on MS medium added with 6 mg/l 2,4-D and subcultured on the low 2,4-D at 0.01-0.1 mg/l obtained embryoids. Its agreed with the report of Sharp et al. (1980) which induced callus and embryoids of oil palm on low or with out 2,4-D medium. The development to be embryoid of Wai Nam Pueng (Calamus sp.) obtained in 30 days different from the report of Ahee et al.(1981) which embryoid of oil palm were obtained for 6-12 months, Pannetier et al.(1981) obtained embryoids for 5-6 months, Tantawan (1988) 150-300 days.

Root induction of Wai Nam Pueng (Calamus sp.) on MS medium added with NAA and IBA 0-16 mg/l. Its appeared that shoot of Wai NamPueng obtained root on MS medium added with 0.25-16 mg/l NAA or IBA. NAA and IBA were auxins that effected for root formation (Leopold 1963). Auxins encorage RNA and protiens synthesis for root formation (Leopold and Kriedemann,1975). Root formation of plants must have relation factors, level of plant growth regulators which suitable in various stage of root deve-

lopment, nutrient accumulations and rooting factors. Shoot of Wai Nam Pueng obtained 90% root formation on MS medium added with 0.5 mg/l NAA or 8 mg/l IBA which appropiate for rooting of this species.

Culturing of young inflorescence of Calamus sp. developed to be young flower calli and embryoids on the same medium because of 1-20 cm length of young inflorescence which different age of differentiation to be inflorescence. Differnt age and length of young inflorescence effecton development of inflorescence were different. Thus the explants were different. Explants from apex of inflorescence there were young meristematic tissue which active (Tiamchai 2523). These tissue developed to be callus and embryoids. Explants from inflorescence were differentiated to specific tissue and developed to be young flower. Developing stage of inflorescence were different of the hormone level. On general the young tissue there were more auxin than the old tissue. Thus the inflorescence were differnt response from exogenous 2,4-D. The young flower developed to be embryoids which happen during development of tissue to be embryoid cells. Development of embryoid cells need specific amount of plant growth regulators and conditions (Evan and Sharp 1981). From observation tissue developed to be young flower were old and there were some browning. This experiment was consistent with the research of Smith and Thomas (1973) which culturing of oil palm young flowers. The Inflores cence shorter than 20 cm could develop to be callus and infloresence longer than 20 cm could develop to be flower.

Culturing embryogenic tissue of Calamus caesius Blume.in plas tic plate (24 well plate Costar) by preparing the MS (Murashige and Skoog 1962),Y3 (Eeuwens 1976) and WPM (Lloyd and McCown 1981)(2,3.4) media with various concentration of 0-10 mg/l 2,4-D and 0-6 mg/l BAP. Callus induction on MS medium added with variuos combinations and concentration of auxin and cytokinin such as 2,4-D, IBA, NAA, picloram, BAP, 2iP and kinetin. The results showed that in every combination could induce callus but the selection for the combination and concentrations were 2,4-D+ BAP.

Callus multiplication of Calamus caesius Blume. the explants were calli previous experiment which had cultured onto the MS medium under different conditions appeared that the callus could multiple at 30°C under the dark 24 hrs-daily on MS medium added with 0.5 mg/l 2,4-D + 2 mg/l BAP was the best.

The development of callus on MS medium with 2,4-D and BAP under 16 hrs.-daily light condition showed better result on callus enlargement and shoot formation than under the dark. Callus development to be embryo genesis and shoot formation in 45 days. Shoot multiplication employed 4-5 multiple shoots per piece was was better than using single shoot and multiplication under light condition were better than under the dark condition.

From induction the explants from base of stem of Calamus caesius on MS medium added with 20 mg/l BAP for 3 months appeared that that they were enlarged and obtained multiple shoots. There were vitrification for cultured on this medium. Subculture on MS medium contanined with 0.5 mg/l 2,4-D and 2 mg/l BAP for 2 weekks there were increasing of multiple shoots. Seperated the biggest shoot for root induction on MS medium added with 0.2 % activated charcoal obtained root

in one month. This experiment appeared that the base of stem were able to induce multiple shoots on high concentration of BAP. From Aziah (1987) culture Calamus manan on MS medium contained with high Cytokinin could induce shoot under the dark condition but our experiments cultured Calamus caesius could induce shoot under light condition. For multiplica tion subculture shoot on MS medium on low concentration of cytokinin. For Calamus caesius were culture on MS medium add with 2 mg/l BAP and could multiple for every 2-4 weeks.

Root formation of Calamus caesius were obtained 100 % for one month on MS medium devoid of plant growth regulator. Auxin in previous medium were enough for rooting. For our study the culturing on the medium without activated charcoal appeared browning and inhibit growth of plant-lets when compared with culture on the medium added with activated char-coal. Browning which were commonly in palm tissue and absorbed by acti-vated charcoal.

Transplanting plantlets from embryo culture amd organogenesis were successful. The plantlets from embryo culture which transplanted were survived for 84.6 % and the plantlets obtained from organogenesis after transplanted were survived for 90.4 %. The 2 months old plantlets from organogenesis would be more vigor than plantlets from embryo cul-ture which were 8 months old. The problem after transplant into plastic bags were drying and some of them were stem rotted at the base and root. Plantlets supplied with sufficient nutrients in a rigidly controlled environment free contamination, would have undergone a shock when trans-ferred to untreated medium. Other facters that could have increased sus ceptibility to fungal infection.

Effect of temperature on growth of Calamus caesius Blume. multiple shoot and seedlings. The Embryoids of Calamus sp. which cultured on MS medium addedwith 0.5 mg/l 2,4-D + 2 mg/l BAP were employed for the experiment. The cultures were incubated at 5°C,10°C, 20°C and 25°C for 60 days in growth chamber under dark condition. Studying effect of low temperature on rattan multiple shoots, in vitro seedling and embryoids. The multiple shoots of Calamus caesius incu-bated at 5°C and 10°C turned brown and die in one week. Seedling could survive and growth very slow at 5°C and 10°C. For the embryoids incu-bated at 10°C there were new and healthy embryoids but at 5°C they grew too slow and were not good embryoids and callus.

- The effects of growth retardant and growth inhibitor and Mixed media on growth of seedling and multiple shoots. Daminozide at 1,500 mg/l produced no increase in height of seedlings.

CONCLUSION

Research to develop in vitro conservation and propagation techniques has been conducted at Plant Tissue Culture Laboratory, The Royal Chitralada Projects and Department of Horticulture, Kasetsart University and success on Wai Nam Pueng (Calamus sp.) and Wai Takra Thong (Calamus caesius Blume.) But on Wai Hom (Calamus pandanosmus Furtado. is not satisfy because of low multiplication rate.

The media which appropiate for rattan plant tissue cullture are:

Wai Nam Pueng (Calamus sp.)

- MS + 500 mg/l casein hydrolysate + 0.3 % activated caharcoal for callus induction and added with low 2,4-D induce embryoids and multi plication on MS + 2 mg/l BAP and 0.5 mg/l 2,4-D. The rooting medium is MS + 0.5 mg/l NAA.

Wai Takra Thong (Calamus caesius Blume.)

- MS + 0.5 mg/l 2,4-D and 2 mg/l BAP for induction and multipli cation. For shoot formation cultured in light conditions. Calamus caesius easy to rooting on MS medium without plant growth regulator.

Wai Hom (Calamus pandanosmus Furtado.

- Culturing of Calamus pandanosmus on various medium, concentration and condition were not satisfy because of low multiplication rate. and unfortunately which this species was extreme rare and difficult to find plant materail. We are try to find the suitable medium for conservation and propagation which we continue the research work.

For conservation

- the effect of temperature on growth of Calamus caesius Blume. multiple shoot and seedlings. The multiple shoots incubated at 5°C and 10°C turned brown and die in one week. Seedling could survive and growth very slow at 5°C and 10°C. For Calamus sp. embryoids the growth of embryoids at 10°C there were new and healthy embryoids.

- The effects of growth retardant and growth inhibitor and Mixed media on growth of seedling and multiple shoots. Daminozide at 1,500 mg/l produced no increase in height of seedlings.

Germplasm collection have been conducted by collection of rattan seedlings which collected from the natural forest and take care in the nursery at the Royal Chitralada Projects and nursery of Dept. of Forest biology Faculty of Forestry, Kasetsart University. Collection by planting in the Diperocarps Demonstration Forest on Chitralada Palace ground. The encoragement for interesting parties in conservation all kinds of rattan species by receiving the official co-operation under the leading of Trang govornor established Trang Rattan Collection Project.

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IMPACT RELEVANCE AND TECHNOLOGY TRANSFER

Impact Relevance

The successful of this project will have economic, scientific, and social impacts to the countries that have rattan resources.

Economic impacts : The large volume in rattan trade demonstrates that, there is great demand for rattan products in the world. The rattan industry and trade volume is a multi million dollar business. Rattan furniture and products have been admired by consumers in America and Europe. Rattan is being considered as one of economic crop harvested from the forest.

Scientific impacts : This project is a new exploration in the use of tissue culture for rattan conservation and mass propagation. This approach will be adopted for rattan germplasm bank to be established in Thailand and eleswhere. Rattan around the world and the native ones will be conserved in vitro. The collected germplasm will served as genetic resources for future rattan improvement. Rattan material will be available for the plant breeder to employ in breeding programs. Perfection in tissue culture for rattan would mean that the problem for inadequate seed supply of superior cultivars were solved. Also, long distance shipment and international germplasm exchange of rattan including the long term storage of clonal materials will be improved.

Social impacts : The rattan industry is labor intensive and its potential has been considered not only interm of manpower employment, but also for its complete cycle production due to increasing demand in the local market and foreign countries. Rattan industry has a great impact to the livelihood of people in the countryside (Tongacan, 1985). The result of this project meet the increasing demand of large scale plantations, which has an obvious impact on enployment of manpower is benefit to the employment in the rural area, improvement of rural economic. Also, the reforestation program will be more successful because of production of planting stcoks and the economic plants like rattan could be launch under such program and this model will be an example for reforestation program in other countries.

At present there is an increasing interest in improving rattan production in several South East Asia countries and the improved methods should be verified and adopted to increase the rattan cultivation and production.

For the purpose of large scale cultivation purpose which require large numbers of rattan seedlings, plantlets may eventually have to be raised by cloning through tissue culture methods. A tissue culture as proposed in this project with provide a new promissing approach in rattan conservation and mass propagation of three economic rattan species namely Wai Nam Pueng (Calamus sp.), Wai Takra Thong (C. caesius), Wai Hom (C. pandanosmus) of which at present has never been practical anywhere.

Also, the in vitro culture has been proven and employed successfully for genetic conservation and mass propagation in many crops (Sharp,1984). The knowledge gained will be exchanged with other countries in form of publication, conference seminar or personal communication.

Technology transfer

Plant Tissue Culture Laboratory had transfered technology to other government agencies such as:

1. Songkhla Rubber Research Center, Rubber Research Institute, Department of Agriculture, Ministry of Agiculture and Cooperative requested to His Majesty the King of Thailand through Lord Chamberlain for rattan tissue culture technique. The Royal Chitralada Project gave the Rattan Plant Tissue Culture Protocol for research and development of the Songkhla Rubber Research Center.

2. Mahasarakam Plant propagation Center, Royal Forestry Department at Mahasarakam province send the Forestry officer to train Rattan Tissue Culture Technique at Plant Tissue Culture Laboratory, The Royal Chitralada Project.

3. Trainning on Rattan Tissue Culture for Research and Trainning on Agriculture Institute, Rajmankala Institute of Technology, Sakon- Nakorn Province at The Royal Chitralada Projects and collaboration on Edible rattan project.

The project's impact on individuals laboratories, department and institute

Impact of Rattan Project of the Royal Chitralada Projects

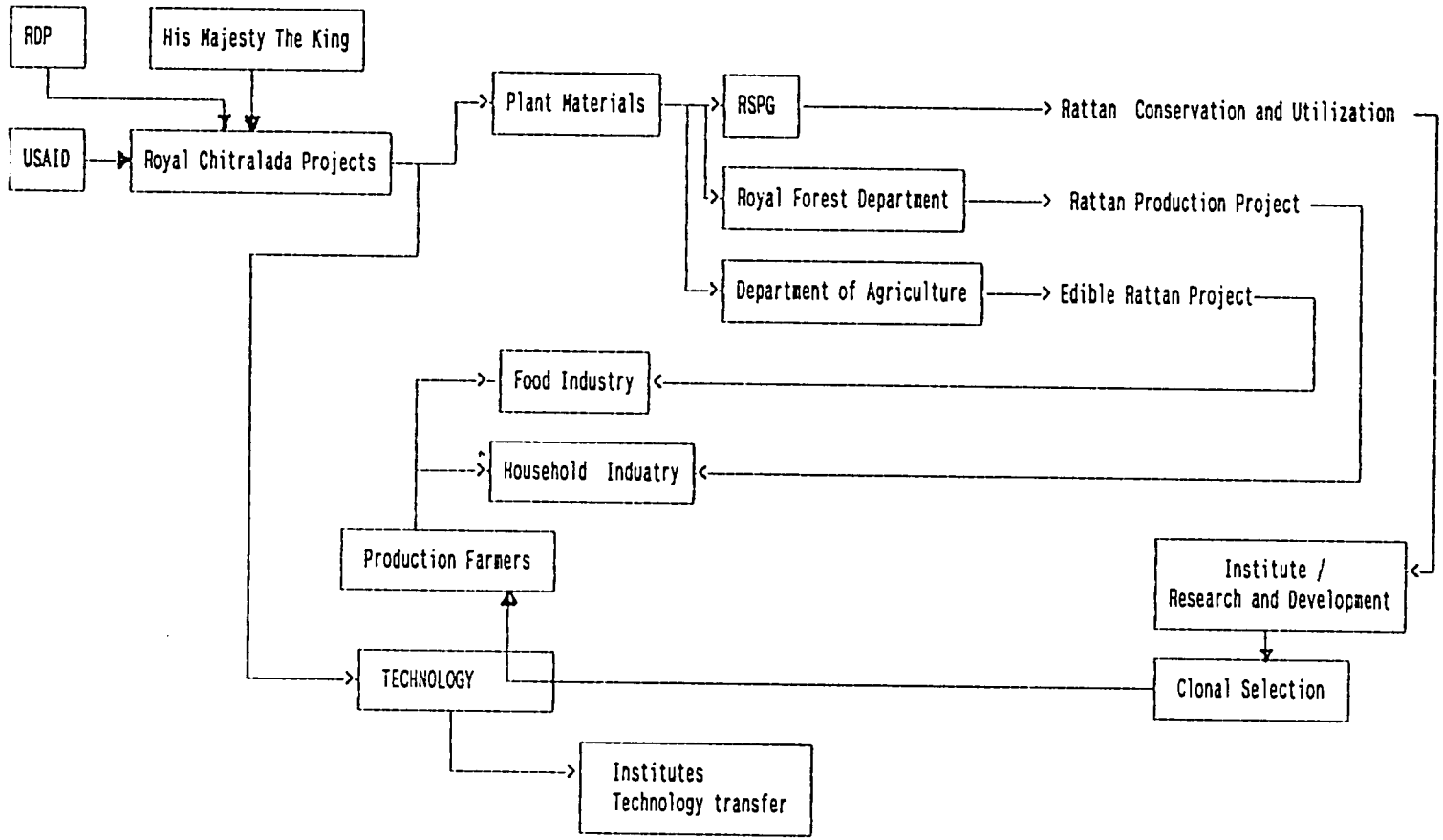
According to the royal initiated of His Majesty the King about Rattan Royal Development project which estabilised Rattan planting at Sukirin, Narathiwat province, southern part of Thailand in 1976. Until after the first Rattan Seminar in Thiland in November 1986. The Royal Chitralada Projects was presented Studying on Rattan Tissue Culture which was the first research on Rattan Tissue Culture. The Royal Initiated established Rattan Research Projects in The Royal Chitralada Projects which supported by The Royal Development Project Board in 1987. And USAID granted for this rattan project had the impact for intersting and encorage the other agencies to studying on rattans.

1. The Rattan Project of the Royal Chitralada Projects had been cooperated with Plant Genetic Conservation Project as Royal initiate of The Crown Princess Maha Chakri Sirindhorn . Rattan are 6 genera of 226 genera which the forestry and plant experts were suggested to conserve urgently and established the cooperation between the other government agencies, Universities and Institutes for conservation and utilization.

2. To encorage the Royal Forestry Department intersting on rattan reserach and established the rattan production for cottage industry.

3. To cooperate with the Department of Agriculture, Ministry of Agriculture and Cooperative on cultivation, cultural practice and management of rattan plantation for industry and edible rattan.

4. The Royal Chitralada Projects transferred rattan tissue culture technique for the other government agencies, universities, institutes, visitors (more than 20,000 visitors/year) and the students which visiting and trainning.



RDP = Royal Development Projects / Office of the Royal Development Projects Board
RSPG = Plant Genetic Conservation Project as Royal Initiation of the Crown Princess Mahachakri Sirindhorn
USAID = U.S. Agency for International Development

Fig.14 Impact of Rattan Project of The Royal Chitralada Projects

The Project made the differance .

This project is under The Royal Chitralada Projects of H.M.the King Bhumipol of Thailand, we have received support from other organization and have a lot of impact to other government agencies are interesting on Rattan Research and development.

What new capacity, equipment or expertise will be left behind in the developing country?

The technogoly of Rattan Plant Tissue Cuture on conservation and propagation were conducted in Thailand and there were impact for interesting and encorage the other agencies to studying on rattans. The resuIt of the project will be collaborating on research and development of Rattans in Thailand and the Asian countries.

The scientific capabilities or collaborating country scientists have been improve

The Royal Chitralada Projects established the Rattan Project by the Royal initiation of His Majesty the King Bhumipol which supported by the Royal Development Projects Board and U.S. Agency for International Development. Its improve the scientist of Plant Tissue Culture Laboratory of The Royal Chitralada Projects for the research work. There are collaborating with other laboratoies and agencies to share knowledge and technology transfer. It will be improve in research and development of rattan species.

PROJECT ACTIVITIES/ OUT PUTS : List meeting attended and held for entire project. List trainning publications patents for entire project

Presentation

1. Mr.Pornchai Chuthamas presented the research work of the project entitle " Callus induction and Shoot formation of Wai Taka-Thong (Calamus caesius Blume.) on 7 th Conference on Methodological Techniques in Biological Science, November 15-16,1989 atCentral Laboratory and Nursery Complex, Kasetsart University, Kampeangsan campus, Nakornpathom province.

2. Mr.Pornchai Chuthamas presented the research entitle "In vitro Conservation and Propagation of Three Economic Species of Rattan" on Tissue Networking/Workshop which organized by BOSTID during July 16-20, 1990 at University of California, Davis, U.S.A.

3. Mr.Pornchai Chuthamas presented the research entitle "In vitro Conservation and Propagation of Calamus caesius Blume on Workshop on AID/SIC Funded Research in Agricultural Biotechnology which organized by Kasetsart University and USAID during August 16-18, 1990.

4. Ms.Piyarat Parinyapong, researcher who presented the research work of the project entitle " Clonal Selecion of Taka-Thong (Calamus caesius Blume.) through Tissue Culture " on 8th Conference on Methodological Techniques in Biological Science during November 15-16,

1990 at Central Laboratory and Nursery Complex, Kasetsart University, Kampeangsan campus, Nakornpathom province.

5. Mr.Pornchai Chuthamas presented the research entitle "In vitro Conservation and Propagation of Calamus caesius Blume. on USAID/ BOSTID NETWORKING MEETING FORESTRY - TREE CROPS at Escuela de Agricultura de la Region Tropical Humeda (E.A.R.T.H.), Costa-Rica April 2-8, 1992.

6. Plant Tissue Culture Laboratory, The Royal Chitralada Projects presented Rattan Tissue Culture on Poster session of The 5th Annual Meeting of Thai Society for Biotechnology and The 10th Annaul Meeting of National Center for Genetic Engineering and Biotechnology on the Topic " Biotechnology for Better Quality of Life" which handle by Biotechnology Assocoation of Thailand, National Center for Genetic Engineering and Biotechnology, cooperate with Faculty of Science, Faculty of Agro-Industry and Faculty of Agriculture, Kasetsart University during November 25-27, 1993 at Hilton Hotel, Bangkok, Thailand.

Publication

Chuthamas P. P.Prutpongse, I.Vongkalung S.Tantiwiwat 1991 . In Vitro Conservatiion and propagation of Calamus caesius Blume. paper on Workshop on AID/SIC Funded Research in Agricultural Biotechnology organized by Kasetsart University and USAID August 1990. p.100-107.

Chuthamas P. P.Prutpongse, I.Vongkalung S.Tantiwiwat M.Ngaosuwan P.Prinyapong 1991 . Callus Induction and Plant Regeneration of Wai Takra Thong (Calamus caesius Blume.) through Tissue Culture Kasetsart University Journal (25) p.299-304. (in Thai)

Chuthamas P. P.Prutpongse, I.Vongkalung S.Tantiwiwat 1991 . In Vitro Conservatiion and propagation of Calamus caesius Blume. paper on USAID/BOSTID NETWORKING MEETING FORESTRY - TREE CROPS at Escuela de Agricultura de la Region Tropical Humeda (E.A.R.T.H.), Costa-Rica April,1992.

COLLABORATION, TRAVEL :
Research observation and Collaboration

Mr.Pornchai (Principal investigator travelled to the United State of America in the first period of the project during July 17-August 20,1988 and attended summer course on the Agricultural Application of Plant Tissue.

Prof. Dr. Toshio Murashige (project advisor) at Dept. of Botany and Plant Science, University of California, Riverside on July 18 - August 5, 1988.

Prof. Don J. Durzan (Project advisor) at Department of Environmental Horticulture, University of California, Davis during August 9-13, 1988 .

Prof. D.W.Burger and Prof. A. Dandekar at Department of Environmental Horticulture, University of California, Davis during August 9-13, 1988 .

Dr.Janet Rice	office of AID building, Washington D.C.
Dr.L.D.Owens,	USDA Agricultural Research Center at Beltsville, Maryland during August 15-18, 1988.
Dr.F.A.Hammerschlag	
Dr.R.J.Grisbach	

Site visiting of U.S.Expert

Dr.Maurice Fried from National Academy of Sceince, Dr. Richard Litz from University of Florida and Dr.Robert Griesbach from U.S. Department of Agriculture visited PSTC grantee at The Royal Chitralada Projects to discuss on the progress report of Research with Mr. Pornchai Principal Investigator of the Research project on "In vitro Conservation and Propagation of Three Economic Species of Rattan" on April 30, 1990.

International Travelling

1. Associate Prof.Isara Vongkalung Co-investigator attened PROSEA (Plant Resource of South East Asia) First International Symposium during May 22-25, 1989 in Jarkata, Indonesia.

2. Mr.Pornchai was participated on Tissue Networking/Workshop during July 16-20, 1990 at University of California, Davis, U.S.A.

3. Associate Prof.Isara Vongkalung and Mr. Pornchai Chuthamas visited Singpore and Malasia on Jan 29-Feb 4 1992.

Dr. Chingchai Hanjanlaksana	Riginal Director of IDRC
Prof. Dr.Roa	University of Singpore
Dr.Wong Sek Man	
Dr.Chiang Shiong Loh	
Dr.P.N.Avadhani	

Forest Research Institute Malaysia (FRIM)

Dr. Wan Razali Wan Mohd.	Director of Forest Division
Dr. M. Darus	Plant Tissue Culture Lab
Mr. Zallpatha B. AB. Rahman	FRIM Officer

4. Mr.Pornchai Chuthamas attend on USAID/BOSTID NETWORKING MEETING FORESTRY - TREE CROPS at Escuela de Agricultura de la Region Tropical Humeda (E.A.R.T.H.), Costa-Rica and presented the research entitle " In vitro Conservation and Propagation of Calamus caesius Blume. April 2-8, 1992.

5. Mr.Pornchai Chuthamas visited Plant Genetic Conservation and Germplasm Bank Management at Beltsville gricultural Research Center, Maryland U.S.A. during April 10-15 1992.

Dr.Robert Griesbach	Florist and Nursery Crops
Dr.R.Lawson	Florist and Nursery Crops
Dr.Kathryn K. Kamo	Plant Physiologist
Dr.David Rubino	Plant Genetic
Dr.George A. White	Plant Introduction Officer
Dr.Jimmie Mowder	Database Manager
Dr.Jim Duke	Medicinal plants Expert
Dr. Mike Fayst	Pomology
Dr.Roy Turner	Sugarcane Quarantine
Dr.Calvin Sperling	Plant Exploration
Mr.Walter Denny	APHIS inspector

Suzanne Huett/ Bill Povich Pome Fruits
Sharon Wainshilbaum Potato/Sweet potato
Howard Waterworth/ Ray Mork
Olivia Mageua Plant Quarantine/ Rice
Kevin Donelly Misc. crops
Bruce Parliment/ Jean French Rice Quarantine

Are large scale will be warranted ?

The large scale will be warranted in the next project which will work under Plant Genetic Conservation Project as a Royal initiate of the Crown Princess MahaChakri Sirindhorn. We transfer technology and cooperate with other government agencies and institutes to work on the Royal Rattan Development Project. We will cooperate with the Department of Agricultural Extension, Ministry of Agriculture and Cooperative to distribute to the farmers.

PROJECT PRODUCTIVITY:

This project success on Wai Nam Pueng (Calamus sp.) and Wai Takra Thong (Calamus caesius Blume.). But on Wai Hom (Calamus pandanosmus Furtado.) is not satisfify. The multiplication rate is very low which will be studying on appropiate media. The cause of unsuccess on this species to be result of extreme rare. The research of rattan will be continue on The Plant Genetic Conservation Project as a Royal Initiate of The Crown Princess MahaChakri Sirindhorn.

To date there are multiple shoots of Calamus sp. foy 14,400x 10 shoots in the incubating room at Plant Tissue culture Laboratory. Multiple shoots on MS devoid of plant growth regulators for 520x3 shoots. Single shoot for root induction 1700 shoots and in vitro seedling 1,000 plants. Seedling in the nursery 1,877 plants. The seedling which deliver for Department of Agricultural Extension for experiment at Chumporn province 3,274 plants. Royal Study Center at Cheingmai province for 1,800 plants, and other govern agencies 1,500 plants.

Calamus caesius Blume. from in vitro seedling collected in the laboratory for 200 clones. and in the nursery 19 clones, and deliver for Department of Agricultural extension 182 plants. And for experiment at Narathiwat Rubber experiment Station, Rubber Research Institute, Department of Agriculture, Narathiwat Province 600 plants.

Calamus pandanosmus Furtado. were collected in the nursery for 40 plants. The seedling which deliverfor Department of Agricultural Extension for experiment at Chumporn province 80 plants.

FUTURE WORK :

From supporting of USAID for this project produce the impact which considered to had a royal initiation of the Crown Princess on Plant Genetic Conservation Projects. Rattan Conservation was an activity which transfer technology from rattan research work to extens and cooperation with the government agencies, universities and other institutes. The Royal Chitralada Projects by The Plant Genetic Conservation Project will be an exist and encorage future rattan reserach and development which have the priorities such as :

1. Survey existing resources :
   - to establish the taxonomic and resource base and the rate of resource depletion;
   - to document and use indigenous knowledge about rattan;
   - to identify critical areas and under utilized species that could be brought into use.

2. Germplasm collection, storage, exchange and characterization :
   - to expand the extent of living collections of rattan;
   - to ex[lore the existing natural genetic diversity which is already at risk of depletion;
   - to screen lines for adaptability to various ecological conditions, suitability for cultivation, and utility of diversified products.

3. Development of propagation techniques:
   - to permit the large-scale production of superior planting material for establishing plantations;
   - to overcome the acknowledged difficulty of obtaining adequate supplies of seed.

4. Investigation of technologies for plantation cultivation:
   - to identify and test cultivation and management techniques for cultivating rattan economically at village level and on comercial scale.

5. Evaluation of domestic use :
   - to quantify the value of domestic (urban and rural) use and of employment generated.

6. Improved harvesting system, use and marketing:
   - to explore opportunities for developing appropiate techniques for harvesting and processing including post-harvest protection, for improved use added value products for domestic and international markets.

Now the Plant Tissue Culture Laboratory, The Royal Chitralada Projects and Plant Genetic Conservation Project as a Royal initiation of the Crown Princess MahaChakri Sirindhorn has the collaboration with other government agencies and laboratories such as :

1. Royal Forestry Department

Silvicculture division on Minor forest product, Royal Forestry Department, Ministry of Agriculture and Cooperative will survey and collect and studying on survey existing resources of rattans which cooperate with Forest-biology Department, Faculty of Forestry, Kasetsart University for classification of rattans.

2. Department of Agriculture, Ministry of Agriculture and Cooperative :

Songkhla Rubber Research Center, Rubber Research Institute, Department of Agriculture at Had yai, Songkhla Province will studying on development of nutrient formula and appropiate media for Rattan Tissue Culture.

Narathiwat Rubber experiment station, Narathiwat Province, Rubber Research Institute, Department of Agriculture will studying on cultivation, cultural practice and management of rattan plantation.

Some of Research Centers and Experiment Stations of Department of Agriculture will collect some rattan species.

3. Kasetsart University

Department of Horticulture, Faculty of Agriculture, Kasetsart University by Assistant Prof. Pranom Prutpongs will collaborate on rattant tissue culture research.

Department of Forest-Biology, Faculty of Forestry, Kasetsart University by Associate Prof' Dr. Issara Vongkalung will calaborate on rattan survey and collection for further research.

4. Plant Tissue Culture Laboratory, The Royal Chitralada Project Studying and developing on nutrient formula and appropiate media for mass propagation for plantation.

Studying on Rattan Identification through Electrophoresis techniques.

Studying on cell suspension culture and regeneration of rattan which collaborate with Kasetsart Agricultural Production Institute (KAPI) and Plant Tissue Culture Unit of Central Scientific Equipment and Laboratory (Central lab), Kasetsart Research and Development Institute (KURDI), Kasetsart University.

5. Research and Training on Agriculture Institute, Rajmankala Institute of Technology, Sakon- Nakorn Province received rattan mass propagation technology on Edible rattan project. And studying on cultivation, cultural practice and management of Edible rattans.

6. Nutritional Research Institute of Mahidol University will studying on nutrition of Edible Rattans.

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ANNEX

BOTANICAL CHARACTERISTIC OF RATTANS

Wai Takra Thong (Calamus caesius Blume.) หวายตะค้าทอง

Clustering medium-sized rattan, climbing high into the canopy with stems ultimately to 100 m. or more in length. Clump very close. Stem without sheaths variable but 8 mm. diameter, with sheaths to 15 mm. in diameter. Internodes 20 cm. long, polished. Sheath dull green armed with sparse triangular pale spines to 25 mm. long by 4 mm. wide, covered with sparse grey indumentum and ometimes with scattered brown scales. Minute spinules 3 by 2 mm. sometimes present between the spines. Knee prominent. Ocrea brownish yellow when dry inconspicuous leaf to 1.0 long, petiole very short or sometime absent in mature stems; cirus to 75 cm. Leaflets to about 9-12 on each side of the rachis, arranged irregularly, usually in alternate pairs occassionally in 3's; leaflets darkgreen above, bluish -white indumentose below, the longest to 30 cm. long by 5 cm. wide, usually cucculate, somewhat plicate, 3 major ribs with clear transverse vine.

Fig.15 Calamus caesius Blume.

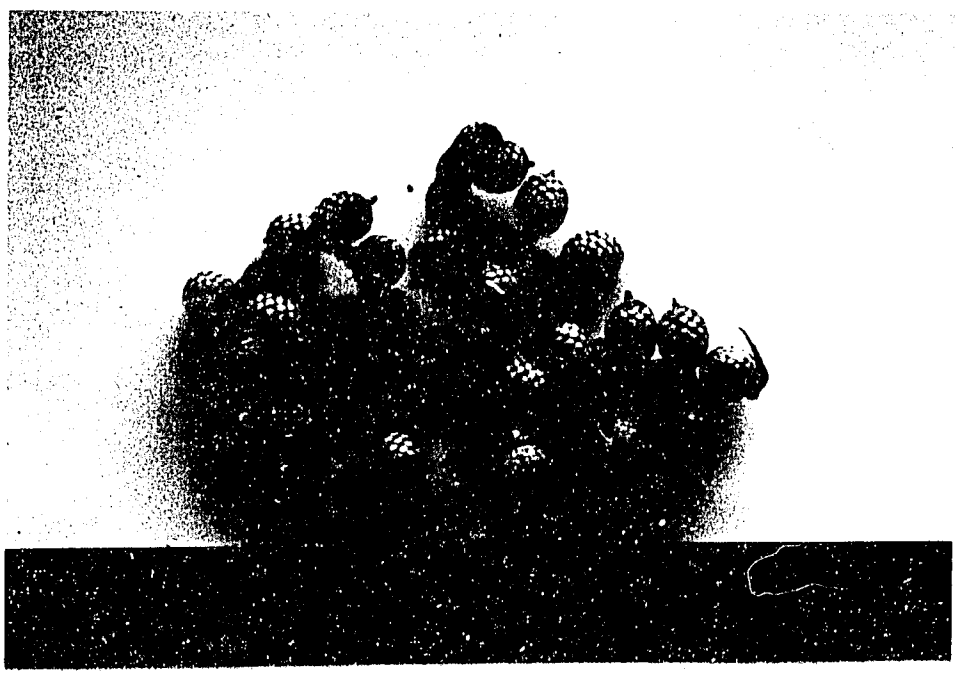

Fig.16 Fruits of Calamus caesius Blume

Wai Nam pueng (Calamus sp.) หวายน้ำผึ้ง

Solitary massive high climbing rattan reaching more than15 m. Stem wiyhout sheath to 3.5 cm. in diameter, with sheathsto 5 cm. in dia meter, internode to 35 cm. long. Sheath dark green armed with sparsely green claw shape spines 2 cm. long by 1 cm wide and sometimes with small brown indumentonse between; spines slightly reflexed. Knee conspicuous. Ocrea greenwish brown and conspicuous. Flagellum to 5 m. long armed with greenwith black tippes claw shape spines. Leaf ecirrate to 3 m. long, petiole to 1 m. long by 25 cm. wide, spearsely armed as is the rachis with triangular shape spines 0.3 by 1.2 cm. on the side and beneath. Leaflet irregular in juvinile leaves, regular in mature leaves, more than 40 on each side; darkgreen, regularly arranged alternate or opposite, 50 cm. long 2.5 cm. wide will small bristles along the leaf margin with 0.5 by 0.8 brown indumentum below but clear above, with 8 parallel veins.

Fig.17 Shoot of Calamus sp.

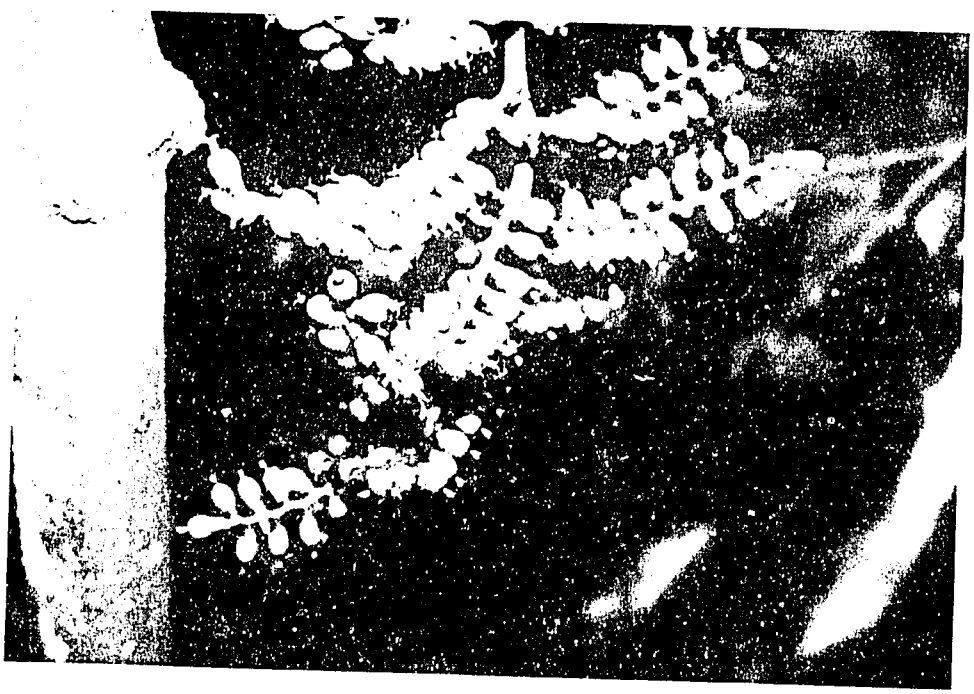

Fig.18 Fruits of Calamus sp.

Wai Hom (Calamus pandanosmus Furtado.) หวายหอม

Small clustering slender rattan climbing to 10 m. or higher; stem without sheaths about 0.4 cm. in diameter, with sheaths to 0.8 cm.; internode to 15 cm. Sheath darkgreen, greenwish yellow when dry, armed with small scattered bulbOus swellings with black tipped spines. Knee inconspicuous. Ocrea small and inconspicuous. Flagellum to 70 cm., with claw shape black tipped 0.20 cm. long irregular arranged spines. Leaf ecirrate and lacking petiole, to 25 cm., with 6 leaflets on eachside of the rachis. The terminal pair joined, other leaflets alternate but irregular arranged, darkgreen 17 by 1 cm. long, upper and lower surface clear, 3 parellel vein with unclear cross vein faintly pandon scented when crushed.

Fig.19 Calamus pandanosmus Furtado.

Fig.20 Inflorescence flowers of Calamus pandanosmus Furtado.

Fig.21 Shoot of Calamus pandanosmus Furtado.

Cell suspension culture of Wai Takra Thong (Calamus caesius Blume.)

Material and Method

Transfer callus from embryo culture in MS medium with 1 mg/l picloram to suspension cullus medium (MS liquid medium which added with 2,4-D or picloram at 0,1,5,10,15 and 20 mg/l) and select the appropiate media that give the highest number of cell per volume, packed all volume and all viability, after that transfer cell suspension to the same media but change the concentration of sucrose to 3 levels (30,40 and 50 g/l of sucrose) and select the media that give the highest number of all per volume, packed cell viability after that tranrfer cell suspension to the same media but added with 0,100,200,300 and 400 l-glutamine of caesein hydrolysate.

Result

Cell suspension culture of Wai TakaThong in Murashige and Skoog (MS) liquid medium which supplemented with 2,4-D of picloram at 0,1,5,10, 15 and 20 mg/l. The result showed that 5 mg/l 2,4-D or 20 mg/l picloram the highest number of cell per volume, packed cell volume and cell viability. In MS medium added with 5 mg/l 2,4-D or 20 mg/l picloram and 30, 40 and 50 g/l of sucrose, every concentration of sucrose showed the tendency to increase number of cell per volume and packed cell volume. But 50 mg/l sucrose gave the highest cell viability. Cell suspension was cultured in MS liquid medium supplemented with 5 mg/l 2,4-D or 20 mg/l picloram 50 g/l sucrose and 0,100,200,300 and 400 L-glutamine mg/l. The results showed that 400 mg/l L-glutamine gave the highest number of cell per volume, packed cell volume and cell viability. When cell suspension of Wai takathong was cultured in MS liquid medium addedwith 5 mg/l 2,4-D or 20 mg/l picloram, 50 g/l sucrose and 0,100,200,300 and 400 mg/l casein hydrolysate. It was found that the cell viability was increased when MS liquid medium with 5 mg/l, 2,4-D 50 g/l sucrose and 200 mg/l casein hydro lysate were used. But the highest cell viability was found when 20 mg/l picloram, 50 g/l sucrose and 300 and 400 mg/l casein hydrolysate were used in MS liquid medium, and casein hydrolysate showed the result better than L-glutamine. The suitable medium for culturing cell suspension was MS liquid medium supplemented with 20 mg/l picloram, 50 g/l sucrose and 300 mg/l casein hydrolysate incresed number of cell per volume, packed cell volume and cell viability.

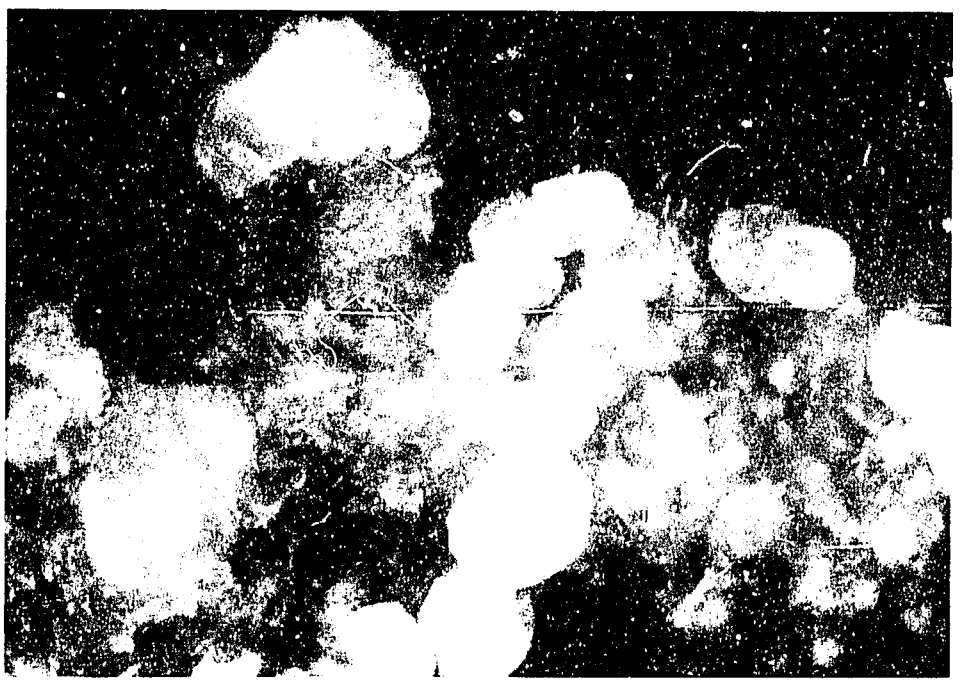

Fig.22 Cell suspension culture Calamus caesius Blume.
for 400 X under Fluoresence Microscope.

Use of Isozymes for Classification of Rattans

Isozymes are useful markers in genetic and breeding researches applications of isozyme studies are in plant classification as of genetic mapping, analysis of genetic structure in plant poppulation, monitoring recombination in remote crosses, identification of somatic cell hybrids, phytogenetic relationship among different species etc. Indentification of specific isozymes could be done either from tissue culture of plant or plant parts. At present we are studying isozyme techniques for classification species and effects could be done to locations and also from somaclonal variation from tissue culture that could be different for zymograms.

Materials and methods

The use of leaves of young seedlings from tissue culture which are 5 species of rattans Wai Nam pueng (Calamus sp.), Wai Takra Thong (Calamus caesius Blume.), Wai Hom (Calamus pandanosmus Furtado.) Wai Kho Dam (Calamus manan Miq.) and Wai ngeuy (Calamus perigrinus). The leaves (in the same weight) were ground to be powder in mortar and pistil with liquid Nitrogen to fine powder and added extraction buffer about 30 mcl/ 1 mg of leaves, mixed and take up all materials in a ependrof tube (Can keep in - 20°C before use).

We used vertical polyacrylamide gel electrophoresis (9% polyacrylamide concentration) was performed as described in detail by labora tory manual LKB 2001 vertical electrophoresis (1982). and observed phenotypes for 4 enzyme systems Z peroxidase, esterase, Leucine aminopeptidase and Phosphogluconate dehydrogenase).

Results

The observed zymogram patterns are illustrated in Figures.

Peroxidase (POD)

We observed 2 or 3 widely saperated zones of intense staining, genetic analysis revealed that each zone was controlled by one gene locus and identified two active alleles at the POD-A and POD-B locus.

Esterase (EST)

We observed 2 or 3 widely saparated zones of intense staining. We identified 2 or 3 active alleles at the EST - A locus, EST- B locus.

Leucine aminopeptidase (LAP)

We observed one saparated zones of intense staining and identified one active alleles at LAP- A.

6-Phosphogluconate dehydrogenase (PGD)

We observed 2 widely saparated zones of intense staining. There are so weak bands between both zones appeared. We identified two active alleles at PGD-A and PGD- B Loci.

Conclusion

This experiment was the first observation on isozymes of rattans. we tried to find the best of extraction buffer and conditions for running electrophoresis. There are so many ploblems which will be solved.

The results can demonstrate polymorphism and chromosomal lacation of genes encoding isozymes makes them helpful as genetic markers of rattan. And we try to improve in materials and methods for the best result in the future.

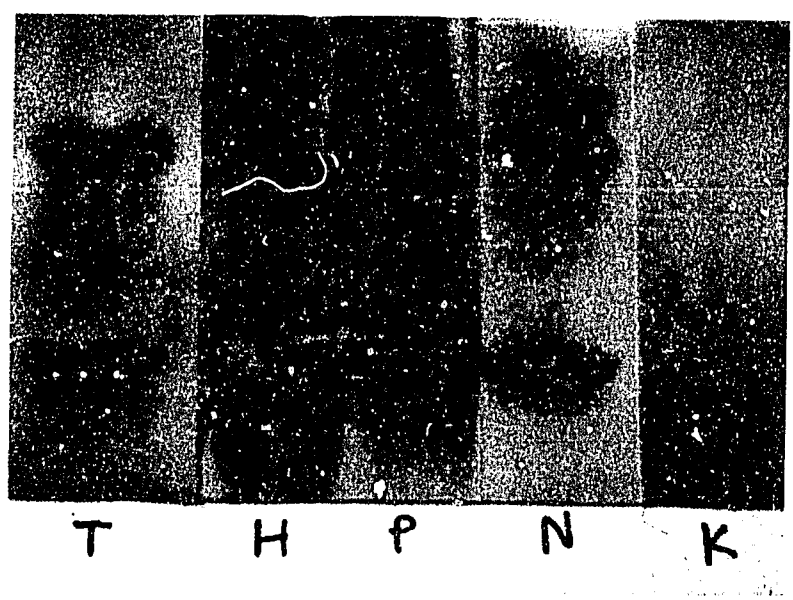

Fig.23 Isozyme pattern of Rattans on Enzyme Peroxidase.

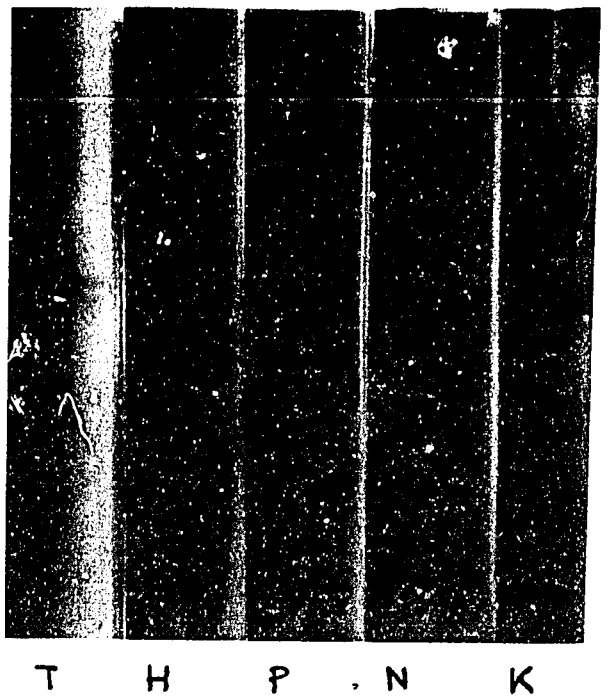

Fig.24 Isozyme pattern of Rattans on Enzyme Esterase.

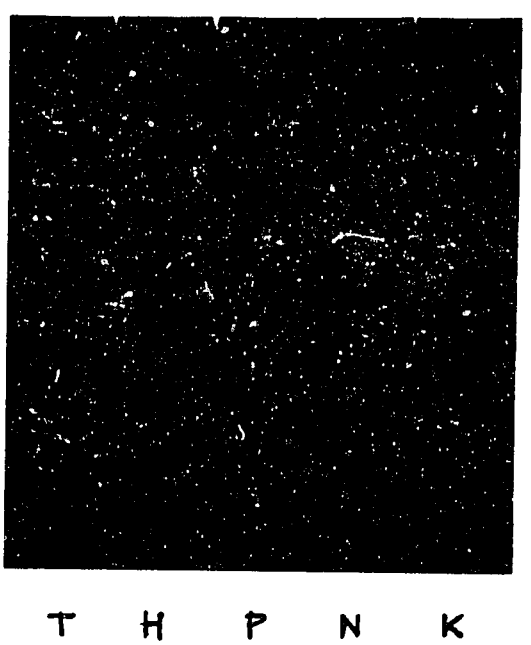

Fig.25 Isozyme pattern of Rattans on Enzyme Leucine aminopepsidase.

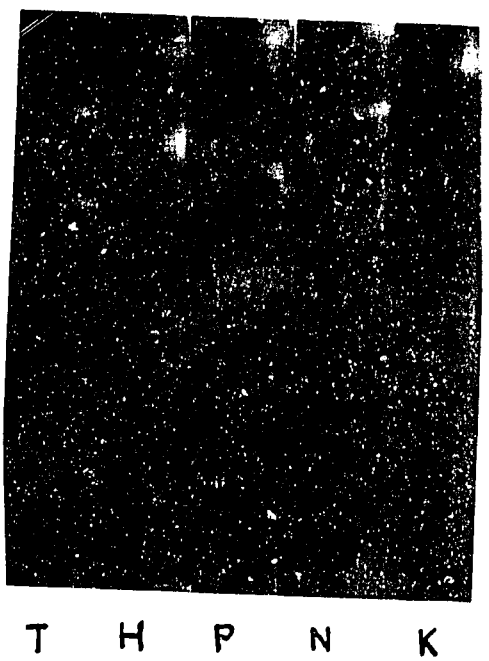

Fig.26 Isozyme pattern of Rattans on Enzyme 6-Phosphogluconate dehydrogenase.

Rattan Protoplast isolation

We used rattan cell suspension culture, First concentrate the cell suspension by gently centrifuging the culture (800-1,000 rpm) and then removing supernatant (0.2 g of cell suspension), Add and equl volume of the protoplast enzyme (Isolation solution) to cincentrated cell suspension and incubated the mixture on a shaker (50 rpm) at 25° C about 7 hours.

Isolation solution	
- Manitol	0.5 M
- Cellulase R-10	10.0 mg/ml
- Macerozyme R-10	10.0 mg/ml
pH	5.5

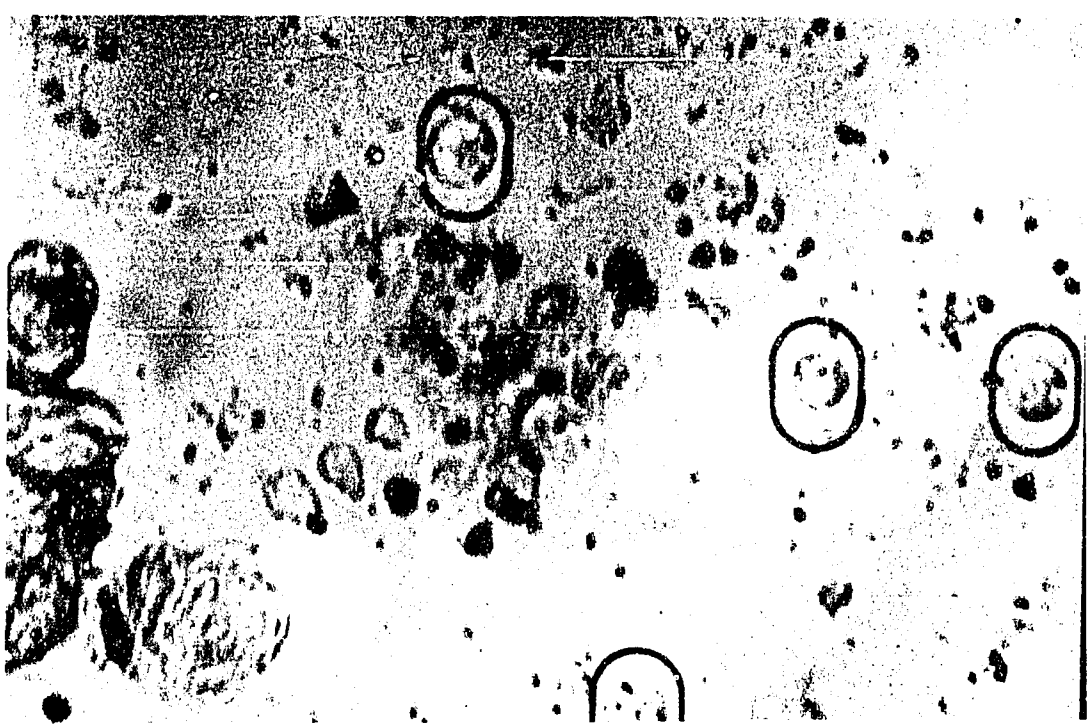

Fig.27 Protoplast of Calamus caesius Blume. for 400 X under Phase Contrast Microscope.

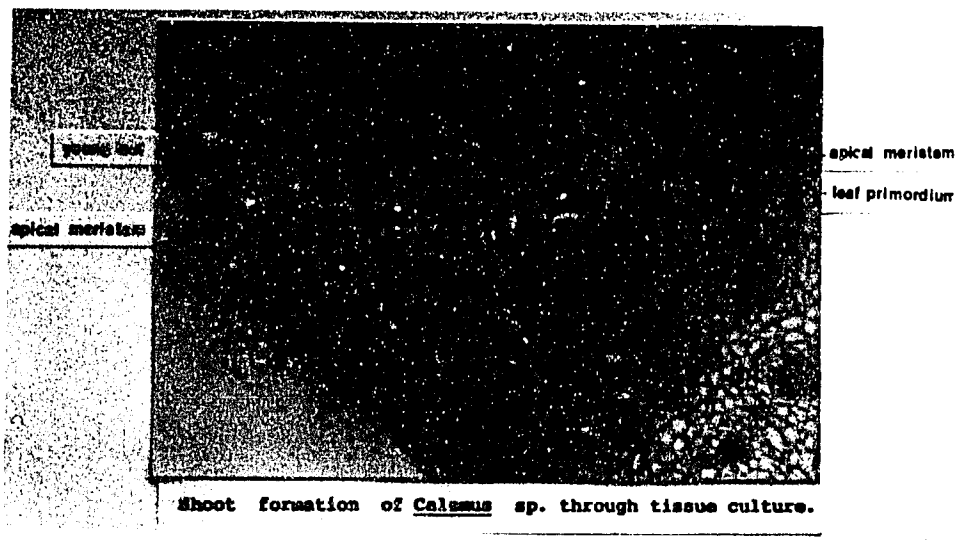

Fig 28 Shoot formation of Calamus sp. through tissue culture

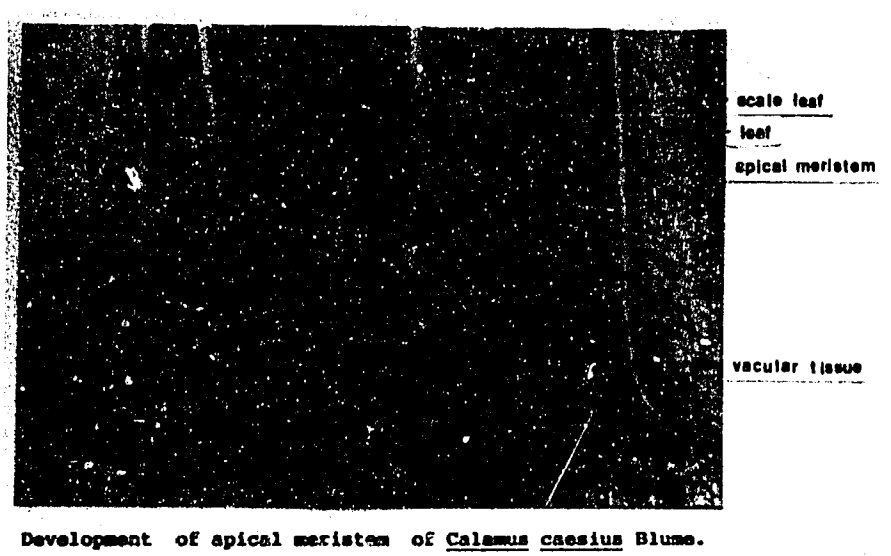

Fig.29 Development of apical meristeem of Calamus caesius Blume. through tissue culture

Fig.30 Experiment on Calamus caesius Blume. Planting on growth and yeild of Rubber in Narathiwat province.

Fig.31 Experiment on Calamus caesius Blume. Planting with Acacia mangium, Tree for its climbing on growth and yeild of Rubber in Narathiwat province.

Fig.32 Experiment on Calamus caesius Blume. Planting with Coffee, Tree for its climbing on growth and yeild of Rubber in Narathiwat province.

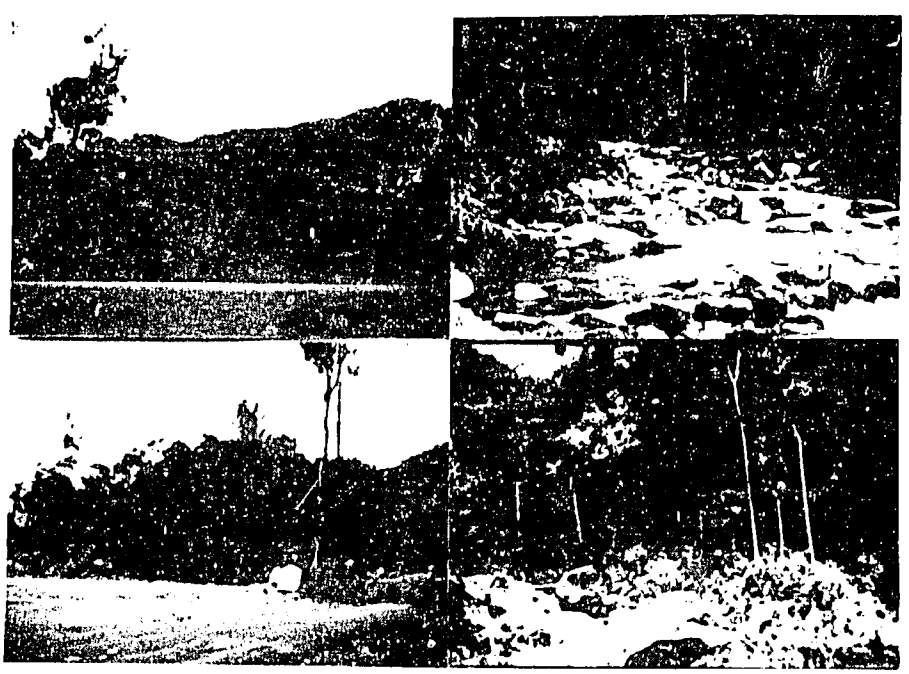

Fig.33 Trang Rattan Collection project near Ton tae waterfall, Palian district, Trang province.

Local Travelling

List of local travelling on rattan surveying and collection—

Trip	Place	Time	Who	Activities
1.	Chumporn province *	Oct. 20-24,1988	Dr.Isara Vongkalung	Survey and collect *C.pandanosmus*
2.	Trang province *	Nov. 14-17,1988	Ms.Manuwadee Ngoasuwan Ms.Piyarat Parinyapong	rattan survey and collection
3.	Trang province *	Jan. 15-17,1989	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection
4.	Narathiwas province*	Jan. 18-22,1989	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection
5.	Kao Yai National Park	Feb. 3 ,1989	Prof. Don J. Durzan Associate Prof.Dr. Isara Vongkalung Assistance Prof. Pranom Pruipogse Mr.Pornchai Chuthamas	rattan survey at Kao Yai Nation Park
6.	Khon Kaen Province	Feb. 4 ,1989	Prof.Don J. Durzan Mr.Pornchai Chuthamas Dr.Sureeya Tantiwiwat	visited Tamarind and Mango project Khon Kaen University
7.	Trang Province *	May 1-4, 1989	Dr. Isara Vongkalung Mr.Pornchai Chuthamas Dr.Sureeya Tantiwiwat	Meetig at Trang Province to established Trang Rattan Collection Project and rattan survey
8.	Trang province *	Jun.1-3,1989	Mr.Pornchai Chuthamas Dr.Isara Vongkalung Dr.Jarunee Vongkalung	join the ritual of rattan painting of Trang Rattan Collection Project
9.	Chumporn provinve *	June 4,1989	Mr.Pornchai Chuthamas Dr.Isara Vongkalung Dr.Jarunee Vongkalung	rattan survey and collection
8.	Chumporn provinve *	Aug.12-15,1989	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection
10.	Chumporn provinve *	Aug.12-15,1989	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection
11.	Chumporn provinve *	Oct.15-22,1989	Mr.Vibool Boonsongsri	rattan survey and collection

12.	Trang Province *	Apr.4-5, 1990	Dr. Isara Vongkalung Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	Meetig at Trang Province to prepare Trang Rattan Collection Project and rattan survey
13.	Chumporn province *	Apr.6-8, 1990	Dr. Isara Vongkalung Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection
14.	Trang Province *	Jun.8-10,1990	Ms.Piyarat Parinyapong	representative of The Royal Chitralada Projects to join a ritual of year 1990 rattan planting of Trang Rattan Collection Project.
15.	Trang Province *	Oct. 1990	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	Meetig of Trang Rattan Collection Project and rattan survey
	Chumporn province *	Oct. 1990	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection
16.	Petchboon province	Nov .11-13, 1990	Dr. Isara Vongkalung Mr. Vibool Boonsongsri	rattan survey and collection
17.	Chumporn province *	Nov. 14-17, 1990	Mr.Vibool Boonsongsri	rattan survey and collection
18.	Cheingmai province	Dec. 5-12,1990	Mr.Pornchai Chuthamas	rattan survey and survey for rattan plantation
19.	Chumporn province *	Feb. 8-11, 1991	Mr.Vibool Boonsongsri	rattan survey and collection
20.	Chumporn province *	Feb. 22-24, 1991	Mr.Vibool Boonsongsri	rattan survey and collection
21.	Chumporn Province *	Jun.14 , 1991	Hon. Prof. Prachid Vamanondth Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri Ms.Piyarat Parinyapong	rattan survey
22.	Trang Province *	Jun.14-17, 1991	Hon. Prof. Prachid Vamanondth Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri Ms.Piyarat Parinyapong	representative of The Royal Chitralada Projects to join a ritual of year 1991 rattan planting of Trang Rattan Collection Project.
23.	Chumporn province *	Jul. 9-12, 1991	Mr.Vibool Boonsongsri	rattan survey and collection
24.	Chumporn province *	Jul. 22-24, 1991	Mr.Vibool Boonsongsri	rattan survey and collection
25.	Chumporn province *	Aug. 23-26, 1991	Mr.Vibool Boonsongsri	rattan survey and collection
26.	Chumporn province *	Nov. 14-17, 1991	Mr.Vibool Boonsongsri	rattan survey and collection

27.	Cheingmai province *	Jan.19-25, 1992	Hon. Prof. Prachid Vamanondth Ass.Prof. Issara Vongkalung Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	Edible Rattan Survey See Rattan at Huai Hong Krai Development Royal Study Center
28.	Chumporn province *	Feb. 10-13, 1992	Mr.Vibool Boonsongsri	rattan survey and collection
29.	Petchboon province *	Feb. 24-26, 1992	Ass. Prof. Issara Vongkalung Mr.Vibool Boonsongsri Ms.Manuwadee Ngaosuwan Ms.Piyarat Parinyapong	rattan survey and collection
30.	Petchboon province *	May. 4-6, 1992	Mr.Vibool Boonsongsri	rattan survey and collection
31.	Petchboon province *	Jun. 5-8, 1992	Mr.Vibool Boonsongsri	rattan survey and collection
32.	Trang Province *	Jul. 9-13, 1992	Hon. Prof. Prachid Vamanondth Ass.Prof. Issara Vongkalung Mr.Pornchai Chuthamas Dr.Sureeya Tantiwiwat Mr.Vibool Boonsongsri	representative of The Royal Chitralada Projects to join a ritual of year 1992 rattan planting of Trang Rattan Collection Project.
33.	Petchboon provinve *	Aug.21-26,1992	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection
36.	Sokonakorn Province * Nhongkai Province * Nakornpanom Province	Sept. 4-9, 1992	Hon. Prof. Prachid Vamanondth Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey
37.	Chumporn provinve *	Oct. 27-31,1382	Mr.Pornchai Chuthamas Mr.Vibool Boonsongsri	rattan survey and collection

DISTANCE FROM BANGKOK :-

1/ Chumporn province, Southern of Thailand 463 km.
2/ Trang Province, Southern of Thailand 828 Km.
3/ Narathiwas Province, Southern of Thailand 1149 km. from Bangkok.
4/ Kao yai Nattional park, Eastern and North Eastern of Thailand 200 km.
5/ Khon Kaen province, Noth Eastern of Thailand 449 km.
6/ Petchboon Province, Nothern of Thailand 346 km.
7/ Cheingmai Province, Northern of Thailand 696 km.

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