Contract No. 53-32 U4-4-241

REPORT NO. 4

(SEMI-ANNUAL REPORT FOR APRIL-SEPTEMBER 1986)

DEVELOPMENT OF CUT FLOWER INDUSTRY IN THE HIGHLANDS TO REPLACE OPIUM BASED AGRICULTURE

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CONTENTS

SUMMARY

This report is the semi-annual report covering the period of April to September 1986 and it is the fourth report of the project. There are eight papers in this report, two of them on chrysanthemum, three on leatherleaf fern, one paper each on lily and carnation, and another paper on tissue culture propagation technique for cut flowers.

An experiment on chrysanthemum was designed to induce matution by tissue culture technique and by gamma ray. Another experiment concerned a trial on pulsing solution formulas.

Papers on leatherleaf fern deal with the problems in the propagation of this cut-leaf plant. The results obtained from the investigations will be very useful.

The paper on lily is an experiment on protoplast culture which is one method to improve the quality of lily.

Problem of short vase-life of carnation is common and in this report, an experiment is described in order to solve the problem.

Another paper included in this report deals with an attempt to propagate important cut-flower crops in large quantity be tissue culture technique.

INTRODUCTION

Since Thailand has been importing cut flowers from the Netherlands and Japan for many years, the trend in imports is increasing every year. In the year 1983, the value of cut flower imports was about 5,500,000 Baht.

Carnation, tulip, chrysanthemum, lily, gerbera, and alstroemeria are the most popular imported cut flowers. In the meantime, researches on various aspects of cut flower production on the highlands such as carnation, chrysanthemum, gladiolus, gypsophila, statice and gerbera have been done during the past years, and have already proved that they have very high potential to substitute opium production. But the cut flower production is still limited due to many problems, such as cultivation technique for high quality and year-round production, diseases, postharvest handling and marketing. These problems should be solved before going into cut flower industry.

The Royal Project in cooperation with the Highland Agriculture Project of Kasetsart University has been carrying out research on the development of cut flower industry in the highlands to replace opium based agriculture. The project was started in November 1984, supported by the USDA for three years. Plan of work has been set as follows :

1. Survey the quantity of various cut flower species presently imported into Thailand and develop strategies for producing these or similar species of comparable market demand.

2. Select cultivars of various cut flower species which produce well in the highlands paying particular attention to the simplicity of growing and marketing.

3. Maintain pathogen-free stocks of cut flowers through tissue culture and multiply for use by hilltribe growers.

4. Train hilltribe growers in the cultural practices and techniques for growing and marketing cut flowers. Develop extension publications, training aids, and selected materials to facilitate technology transfer.

5. Evaluate market potentials and assist hilltribes in marketing cut flowers.

CUT-FLOWER PROPAGATION BY TISSUE CULTURE TECHNIQUES

The import cost of the stocks or bulbs to produce cut-flowers locally is high. The order must be made in great volume and varieties, some of which might not be suitable to the climate of Thailand. The Project decided to use the varieties already selected and studied by the Royal Project. The stocks of those varieties were experimented, using the tissue culture techniques for mass propagation of disease-free stocks within shortest time.

1. The gerbera propagation by tissue culture technique was conducted at the Institute of Agricultural Technology, Mae Jo, by Ms. Lakhana Pradab using the selected varieties of gerbera from the Royal project, Doi Inthanon, -- Beatrix, Clementine, Appelblossom and Symphony. The flowers, in bud and fully bloom, were cleaned. All the disc floret and ray floret parts were withdrawn. The flower stalk was cut to 1.5-2 cm. underneath the receptacle. The first cleaning was done in 70% alcohol for 2-3 seconds. Then it was washed in chlorox, 10% concentration, for 10 minutes, and 5% concentration for 5 minutes respectively. Finally it was cleaned in the sterile distilled water for 10 minutes. The bract and flower stalk exposed to chlorox were cut out. Then the receptacle was separated from the stalk and divided into four parts. The stalk was cut to pieces of 0.5 cm. long. Those were cultured in the medium of Murashige and Skoog (1962) formula modified by using a portion of micro nutrient at half

of normal concentration plus 0.5 mg/litre IAA and 10 mg/litre kinetin. After two weeks, the parts of the stalk and receptacle grew visibly. Callus could be clearly seen in some pieces. Some were only swollen. One month later when the callus became large, it was divided into two parts and were cultured in the same formula of medium. After two weeks it was found that some callus continued to grow, some become black when the cells shrank and finally died.

2. Lily propagation by using tissue culture technique was conducted at the Ramkhamhaeng University by Mr.Udom Navapanich. He received the bulbils of lily nourished in the sterile bottle from Kasetsart University. For some varieties obtained from the Royal Project the bulbil and scale were sterilized -- Easter lily (Lilium longiflorum) and Elegans lily (Lilium elegans). The scale of the bulbils were separated to be cultured in the Murashige and Skoog (1962) formula added with 1 mg/litre kinetin and 1.5 mg/litre NAA, having the nutrient pH at 5.8, exposed to light at 3,000 lux for 16 hours/day and left in darkness for 8 hours/day, at 27-30°C. The number of bulbs produced up to May 1986 is given in Table 1.

Table 1. Number of lily bulbils produced by tissue culture technique up to May 1986.

Varieties	Bulbils from one scale	Total No. of bulbils
Easter lily, Teppoyuri	4	400
Elegans lily, Bellona	3	500
Chinook	1	50
Connecticut King	4	1,546
Fire Creacker	3	10
Golden Melody	2	30
Lady killer	2	140
Red Night	4	900
Sterling Star	1-2	60
Yellow Blaze	1-2	40

From Table 1, it is evident that the medium formula used in this experiment was effectively workable for the propagation of lily of Connecticut King, Red Night, Bellona and Teppoyuri varieties. The two batches of samplings, 100 each were planted at Ang Kha Noi, Doi Inthanon in May and June 1986. The work must be continued to obtain the most effective formula of medium for the propagation of other varieties.

3. Chrysanthemum propagation by tissue culture technique was conducted at Kasetsart University. The flowers of Chrysanthemum indicum cv "Cremon" imported from the Netherlands and known as "Khai Dao" in Thai by its shape were bought. The node of the flower stalk were washed, sterilized and cultured in the medium of Murashige and Skoog (1962) formula, modified by adding 15 per cent of coconut juice and 30 gram/litre sucrose. It was found that some nodes gave rise to small chrysanthemum plantlets, some grew to be flowers blooming in the bottle with small plantlets growing out of the flower base. From those plantlets, further propagation could be made by changing the medium formula to Murashige and Skoog (1962) added with 20 per cent coconut juice and 40 gram/litre sucrose. Within 20 days. they could grow into shoots having 5 nodes, which could be separated and cultured in another medium. The volume can be increased five times every 20 days. From the lower nodes they could give 3-5 shoots, 5 nodes each. Therefore the volume increased very rapidly. The plants produced with this technique were planted at Doi Inthanon, Ang Kha Noi and Huay Luek. From those disease-free stocks, normal propagation by cutting could be done. It is expected that planting for cut-flower can be done in July 1986.

Figure 1. Tissue culture of lily.

Figure 2. Tissue culture of chrysanthemum

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MUTATION INDUCTION IN CHRYSANTHEMUM BY TISSUE CULTURE TECHNIQUE AND GAMMA RAY

INTRODUCTION

Gamma rays have been used to induce mutation in several kinds of plants such as gerbera (Rongrong, 1985), carnation (Chaichumpol, 1983) and kalanchoe (Surawich, 1983). The mutants can be used in the improvement of shape and quality including the tolerance to diseases and pests, especially in the chrysanthemum propagated by tissue culture technique. The use of gamma ray can shorten time for varieties improvement.

MATERIALS AND METHODS

The chrysanthemum employed in the experiment were Chrysanthemum morifolium 'Doikham' and Chrysanthemum indicum 'Cremon'. The terminal cuttings of the chrysanthemum obtained from tissue culture were washed, sterilized and cultured on the medium Murashige and Skoog (1962) plus 20 per cent coconut juice and 40 gm /litre of sucrose. The plantlets grew well. After 20 days or when the plants developed 5 nodes they were exposed to the gamma ray from the gammator, for the first time at the rate of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 Krad as a random to find appropriate rate. After that the propagation was conducted by cutting into nodes and transferred to the new medium.

It was found that those exposed to the γ ray at 2-10 Krad did not grow, the higher quantity of γ ray made the plants die more. Therefore it could be concluded that the effective rate should fall between 1-2 Krad. Another experiment was conducted by emitting the gamma ray at the rate of 0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 Krad. The chrysanthemum exposed to gamma ray at 0-1.5 Krad grew, but the growth rate declined following the higher rates of γ ray. At 2-4 Krad there was no growth (Figure 1). The radioactive plants were propagated for VM_1 and VM_2 plants by tissue culture technique and node cutting. Some irregularities could be traced in VM_1 and VM_2 such as mosaic leaves, thick leaves, stunt, short stem branching, twin plants, plants grown in clump etc. (Figure 2). The radioactive chrysanthemum propagated into VM_2 plants when growing enough to have about 5 nodes was tranferred from the bottle. The agar was washed away from the root; then it was soaked in the fungicide and then grown in the medium, a mixture of burned rice hull and coarse sand at an equal volume, for one week then transplanted to the plastic bags filled with another mixture of medium -- 1 part of burned rice hull, 1 part of coconut fibre, 1 part of coarse sand and $\frac{1}{2}$ part of manure -- for two weeks. An additional light period between 17:00-21:00 hours was needed to prevent flowering, because chrysanthemum responded to flowering during the period of critical day lenght of $13\frac{1}{2}$ hours. After this the plants were transplanted to the beds at Kamphaensgaen Campus of Kasetsart University. It was expected that the plants would flower during February to March. Study could be made on the effect of the gamma rays on the possible mutation in terms of shape and color of the flowers so that the mutants to the required shape and quality could be selected.

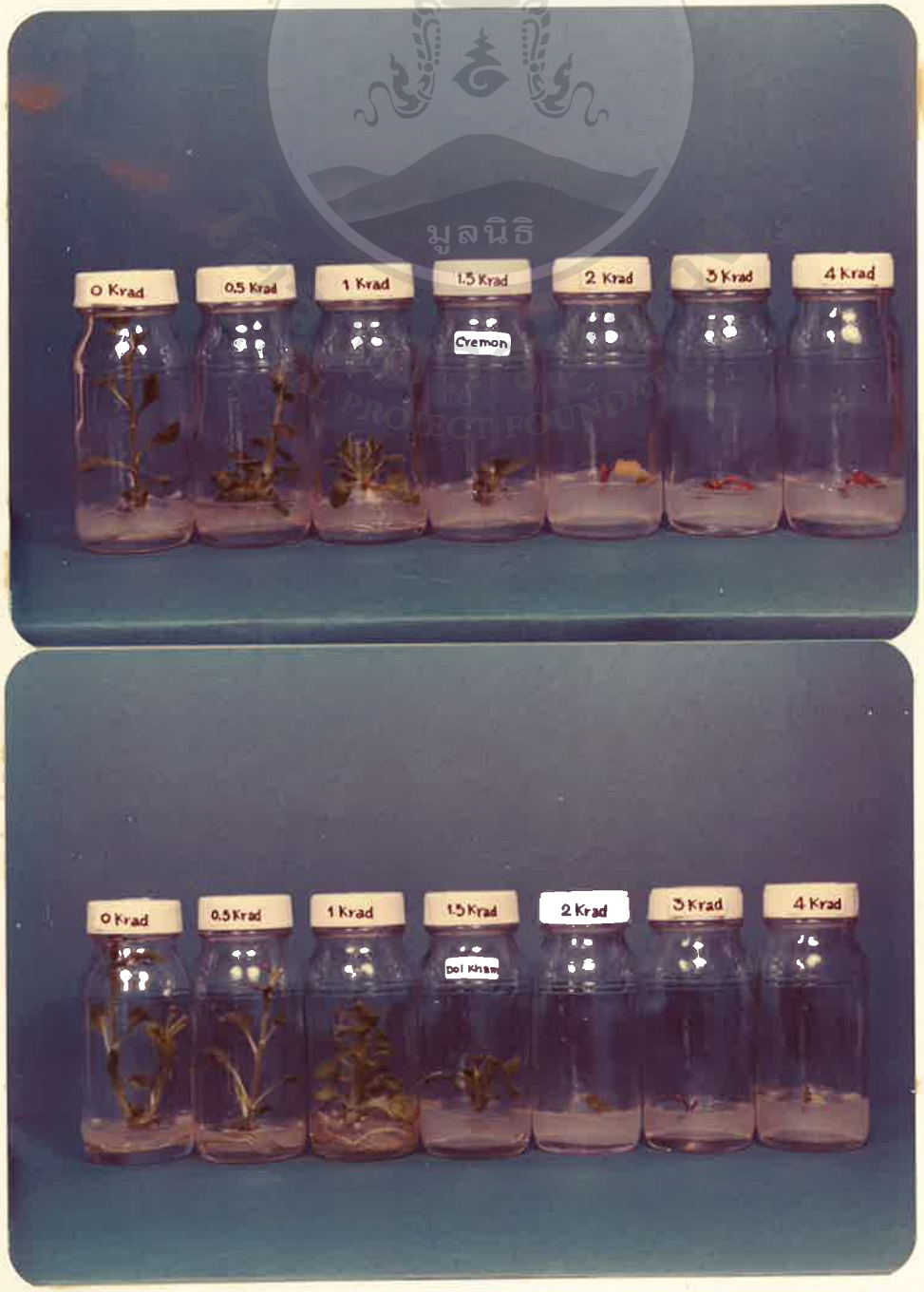

Figure 1. The growth of chrysanthemum plants of both Cremon and Doikham varieties exposed to various rates of gamma ray.

Figure 2. The irregularities of the chrysanthemum "Cremon" (above) and "Doikham" (below) when exposed to 1.5 Krad gamma ray:stunt and thick leaves.

PROTOPLAST CULTURE OF LILY

INTRODUCTION

The protoplast culture of lily was conducted by Assoc. Prof. Boonyuen Kijvicharn of the Department of Biology, Faculty of Science, Khon Kaen University. He received the Lilium Longiflorum tissue cultured stock from Kasetsart University to conduct a research for further improvement of the varieties to obtain the good quality plants.

MATERIALS AND METHODS

Protoplast Isolation Method

The lily plants with the leaves of 5-7 cm. length were selected for protoplast isolation (Figure 1). The lower epidermis was peeled out and the remaining was immersed in the enzyme solution (Figure 2):

Treatment 1	cellulase	1%
	macerozyme	0.5%
	mannitol	9.1%
	pH	5.5
Treatment 2	cellulase	1%
	macerozyme	0.5%
	mannitol	9.1%
	$CaCl_2$	0.1%
	pH	5.5%

They were incubated at the temperature 37°C for 2-2½ hours. The petri dish was inclined allowing the protoplast to accumulate at the bottom. The pipette was used to suck it up and then strained through the sieving cloth. The sieved matter was centrifuged, when the protoplast was isolated, it was cleaned for three times with the washing solution. Then the protoplast was cultured in the liquid medium:-

1. MS + 15% coconut juice
2. Washing solution
3. Nagata & Takebe media (NT)
4. Nagata & Takebe media + 15% coconut juice

RESULTS

The isolation of the protoplast of the lily leaves by immersing in the enzyme solution in both treatments was not different. But the protoplast from treatment 2 could last longer than the treatment 1 (treatment 1 when left for a longer period could easily degenerate). When the protoplast was cultured in the liquid nutrient, the one cultured in the formula Nagata & Takebe plus 15% coconut juice grew well, some parts developed the protuberance and the cytoplasmic strand showing that the cell was active. The cell's activity lasted 7-6 days, then stopped.

Protoplast cultured in the MS formula degenerated within one day; the one cultured in the washing solution was in good condition and could continue for 7-8 days. Inside the cell the

cytoplasmic strand developed and the chloroplast accumulated around nucleus.

Figure 1. The lily plant, the leaves of which are to be processed for protoplast division.

Figure 2. The leaves without lower epidermis immersed in the washing solution.

Figure 3. The protoplast isolated from the leaf part.

Figure 4. The protoplast cultured in the washing solution for 1 day.

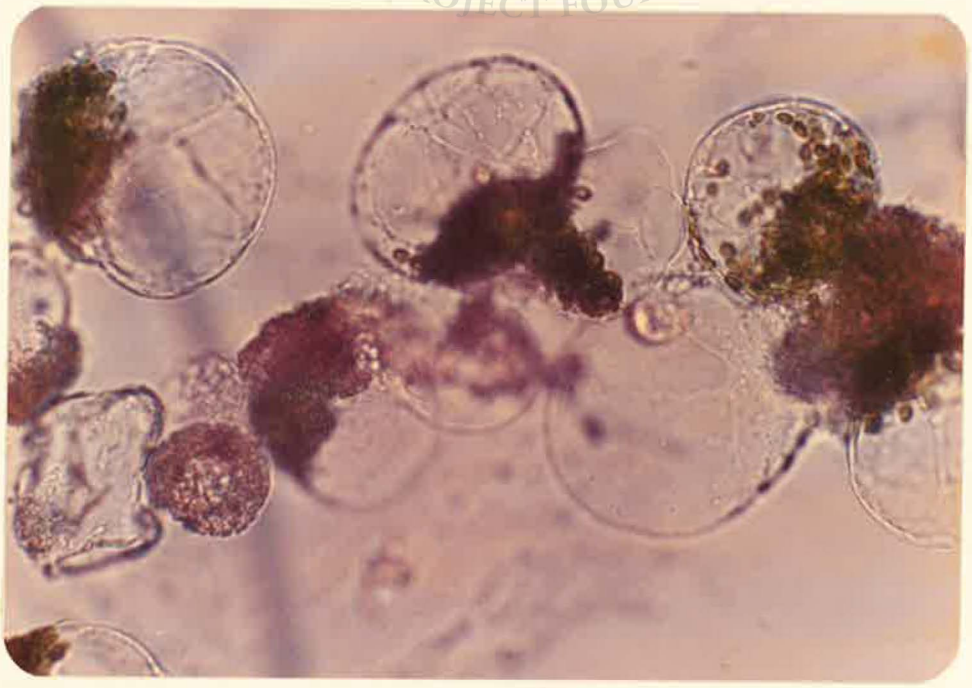

Figure 5. Protoplast cultured in washing solution for 4 days having cytoplasmic strand and chloroplast around the nucleus.

Figure 6. Protoplast having protruberance in the nutrient formula Nagota & Tekebe plus coconut juice 15%.

EFFECT OF PHYSAN-20 AND SUCROSE ON THE VASE-LIFE OF CARNATION

INTRODUCTION

Carnation is a temperate cut-flower and can grow well in Northern Thailand. Although the imported flower is costly, it has still been imported because the imported ones are better than the local product especially the longer vase-life. The short vase-life is therefore another important problem making the local product unpopular. The study has been conducted to find out if application of Physan-20 and sucrose could expand the vase-life of the local carnation.

MATERIALS AND METHODS

The carnation employed in this study was the Red Sim variety grown at Royal Project Inthanon Station, Chiang Mai. The flowers were cut while 80 per cent open, wet-packed in paper box and transported by a car and reached Bangkok within 12 hours. Flowers with good and consistent quality were selected and their stalks were cut to 40 cm. Only 3-4 leaves were left on the stalk. The carnation flowers were pulsed in tap water and solutions at different concentrations for 18 hours at room temperture and placed in the vase filled with tap water -- 3 flowers in one vase with 200 ml. water. The data were recorded about the vase life, number of rotten stalks and the appearance of solution in the vase. The average temperature and relative humidity of the room were 29.5°C and 65.7% respectively.

RESULTS AND DISCUSSION

Physan-20 is a quarternary ammonium compound chemical having the biocide property (Halevy and Mayak 1981) and is widely used to extend the shelf-life of various kinds of flowers commercially grown abroad (Reid 1985). The carnation flowers treated by immersing in the Physan-20 and sucrose lasted longer than those untreated. The carnation flowers treated by immersing in 660 ppm Physan-20 and 5% sucrose lasted longest -- 5.92 ± 1.58 days. The untreated flowers could last only 3.83 ± 1.33 days (Table 1). The reasons might be that the microorganisms on the stalks were gone after pulsing and the flowers absorbed Physan-20 preventing the xylem blocking caused by microorganism (Zagory and Reid 1986). When the flowers were placed in the vase filled with tap water, their stalks were not rotten easily and the solution therein was not so unclear (Table 1). The carnation flowers could absorb more water to replace the loss by transpiration (Halevy 1975). The sucrose contained in the solution also helped extend the vase--life because it was the source of energy in several processes (Nichols 1973). Sucrose also helped reduce the transpiration by closing the stomata (Marousky 1969) and increase the water absorption by balancing the osmotic potential of the carnation flower (Acock and Nichols 1979). Therefore the joint property of Physan-20 and sucrose lengthened the vase-life of the untreated flowers.

Table 1. Vase life, percentage of rotten stalks and the appearance of solution after 18 hours of pulsing in various treatments.

Treatment	Vase life (day)	Rotten stalks (%)	Appearance of solution
Tap water (control)	2.83±1.33	66.67	decayed, slimy, precipitating
Physan-20 200 ppm + sucrose 5%	4.25±0.75	58.33	dull white in color
Physan-20 400 ppm + sucrose 5%	5.08±1.00	91.67	clear and yellow
Physan-20 600 ppm + sucrose 5%	5.92±1.56	33.33	cloudy
Physan-20 200 ppm + sucrose 10%	5.36±1.63	63.64	dull white in color
Physan-20 400 ppm + sucrose 10%	4.54±1.29	81.82	clear and yellow
Physan-20 600 ppm + sucrose 10%	4.60±2.07	90.00	clear and deep yellow

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EFFECT OF THE DIPPING OF CHRYSANTHEMUM STALK IN CHEMICAL SOLUTION BEFORE USING

INTRODUCTION

Chrysanthemum, standard type, was popular among flower lovers. From the survey on the operation of flower shops in Bangkok Metropolis the chrysanthemum, standard type, ranked fourth in popularity among twelve kinds of imported flowers. However, it was both imported and locally grown. The chrysanthemum grown in Thailand in winter had better quality than those grown during summertime (Sompian, 1982). From the study of chrysanthemum grown during winter especially in the Northern provinces such as Chiang Mai it had as good quality as the imported one -- large and bright-colored flower, perfect leaf, large, long and straight stalk. However, the vase-life of the local flowers was short, the petals and leaves quickly wilted and color changed rapidly if kept at the room temperature for only 2-3 days. These problems corresponded to the factors both before and after harvest (Anon, 1980). The factors concerning the post-harvest treatment were packaging, transportation, temperature, humidity and the quality of water used for immersion etc. There were many methods to improve the quality and extend the vase-life of chrysanthemum and one was to dip the stalk in the chemical solution for a short time before displaying in the shop or arranging in the vase (Marousky, 1972).

Therefore an experiment was conducted to apply different formulas of chemical solution with the chrysanthemum to select the best solution for vase-life expansion.

MATERIALS AND METHODS

The chrysanthemum (Chrysanthemum morifolium) employed in this experiment was the Doi Kham Leuang variety grown at Huay Luek Station of the Royal Project, Chiang Mai. The flowers were dry-packed and carried by the truck of the Royal Project. It was a non-airconditioned ten-wheel truck and took about 12 hours to reach Bangkok from Chiang Mai. Selection was conducted for good quality and consistent size of flower and stalk. The flower stalk was cut to about 45 cm. with 10-12 leaves left. The flower stalks were immersed in the tap water 15 cm. up from the tip (Control) and different formulas of solution -- $AgNo_3$ 68 mg./litre + $Na_2S_2O_3$ 794 mg./litre + BA 25 mg./litre + sucrose 5%, Physan - 20 200 mg./litre + sucrose 5% and $AgNO_3$ 50 mg./litre + 8 - hydroxyquinoline sulfate (8-HQS) 200 mg./litre + sucrose 5% for 10-20 hours. Then each flower was put in the 250 ml. flasks containing 200 ml. tap water, 12 flowers for each treatment. The record was taken about the first day the flower or leaves began to wilt, the length of rotten stalk end, water absorption and vase-life. The condition of the experiment room : average temperature and relative humidity were 29.4°C and 69.5% respectively all through the experiment. The crysanthemums were

exposed to natural light about 12 hrs/day. The data obtained were the average of 12 flowers in each treatment.

RESULTS AND DISCUSSION

From the result of the experiment the chrysanthemum of Doi Kham Lueng variety responded differently to various formulas of solutions (Figure 1). The chrysanthemum soaked in the solution $AgNO_3$ + 8-HQS + sucrose had increasing fresh weight, while those immersed in tap water and other formulas the fresh weight decreased after 20 days in vase, except for the ones soaked in Physan - 20 + sucrose for 20 hours and in $AgNO_3$ + 8-HQS + sucrose for 20 hours which gained more fresh weight than the 10-hour immersion (Table 1). Both $AgNO_3$ and 8-HQS had the property of bactericide and reduced the possibility of the xylem blocking in the stalks caused by bacteria (Larsen and Cromarty, 1967, Mayak et al., 1977) enabling the flowers to absorb water better and thus gain more fresh weight. The solution containing $AgNO_3$ and 8-HQS had more bactericide properties than other formulas. The stalks immersed in $AgNO_3$ + 8-HQS + sucrose were not rotten, while in tap water and other formulas the stalks got rotten to a similar length (Table 1) because $AgNO_3$ and 8-HQS coated the outer skin of the chrysanthemum stalk tips, protecting them from the attack of bacteria (Korranek and Paul, 1975).

For the chrysanthemum dipped in $AgNO_3$ + 8-HQS + sucrose solution, the leaves and flower wilted slower than those dipped in other formulas. The ones dipped in this formula for 20 hours, the leaves and flower wilted slower than those dipped for 10 hours (Table 2). The slow wiltness should correspond to the increase of the fresh weight of the chrysanthemum flower which showed that it absorbed water more than transpired (Mayak et al., 1974). Therefore, the flower which absorbed more water would help the bloom and the leaves to last longer. Such slow wiltness of flower and leaves was not due to the anti-transpirant property of $AgNO_3$ and 8-HQS (Gay and Nichols, 1979) but to its bactericide property as mentioned earlier. Sucrose in the solution $AgNO_3$ + 8-HQS + sucrose did not only serve as the nutrient for chrysanthemum (Nichols, 1973), but also helped adjust the balance of water in the flower as they made the stomata close thus reduced the transpiration (Marousky, 1972, Halevy, 1976).

Comparing the length of the vase-life of chrysanthemum immersed in different solutions for 10 and 20 hours, it was found that those immersed in $AgNO_3$ + 8-HQS + sucrose for 20 hours had the longest vase-life -- 7.5 $\pm$ 1.68 days; the shortest were the ones immersed in the tap water for 10 hours, 3.17 $\pm$ 0.39 days (Table 2). The long vase-life should correspond to the property of the $AgNO_3$, 8-HQS and sucrose as aforementioned. Moreover, the flower could absorb more solution than the one with 10 hour immersion. There was a significant difference between the chrysanthemum treated with

the solution $AgNO_3$ + 8-HQS + sucrose and those untreated (Figure 2). However, the 20-hour immersion might be too long for commercial practice. A study should be conducted to see if the immersion period would become shorter when the concentration of each chemical was higher (Halevy and Mayak, 1981), so that the chrysanthemum could be delivered to the market as soon as possible.

Figure 1. Condition of chrysanthemum flowers put in the vase filled with tap water for five days, ater 20-hour immersion in different solutions.

Table 1. The increase of fresh weight and the rot of the stalk tips of chrysanthemum after 10 hour and 20 hour immersion in different solutions.

Treatment	Change of fresh weight after 2 days in vase (g) 1/	Rottenness of stalk tips (cm.) 2/
10-hour immersion		
Tap water (Control)	- 7.56	12.96 ± 1.53
$AgNO_3$ + $Na_2S_2O_3$ + BA + sucrose	- 1.23	12.87 ± 1.56
Physan - 20 + sucrose	- 0.40	11.96 ± 1.21
$AgNO_3$ + 8-HQS + sucrose	+ 2.61	0
20-hour immersion		
Tap water (Control)	- 13.29	11.42 ± 3.33
$AgNO_3$ + $Na_2S_2O_3$ + BA + sucrose	- 7.85	12.79 ± 3.82
Physan - 20 + sucrose	+ 3.93	10.50 ± 8.19
$AgNO_3$ + 8-HQS + sucrose	+ 5.33	0

1/ - decrease + increase

2/ measured after the vase-life was over

Figure 2. Condition of chrysanthemum 5 days in vase filled with tap water after 20-hour immersion in the tap water (left), and 20-hour immersion in $AgNO_3$ + 8-HQS + sucrose (right).

Table 2. Wiltness of petal or leaves and vase-life of chrysanthemum immersed in different solutions for 10 and 20 hours.

Treatment	First day of petal or leaf wilting (day)	Vase-life (day)
10 hour immersion		
Tap water (Control)	2.75 ± 0.45	3.17 ± 0.39
$AgNO_3$ + $Na_2S_2O_3$ + BA + sucrose	3.25 ± 0.45	4.25 ± 0.45
Physan - 20 + sucrose	3.33 ± 0.78	4.75 ± 1.60
$AgNO_3$ + 8-HQS + sucrose	3.92 ± 1.16	5.60 ± 1.61
20 hour immersion		
Tap water (Control)	1.92 ± 0.79	4.17 ± 1.11
$AgNO_3$ + $Na_2S_2O_3$ + BA + sucrose	2.67 ± 0.49	5.33 ± 1.37
Physan - 20 + sucrose	3.75 ± 1.22	5.92 ± 1.38
$AgNO_3$ + 8-HQS + sucrose	5.75 ± 1.82	7.50 ± 1.68

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STUDY OF THE CHARACTERISTICS AND METHODS OF COMMERCIAL SCALE PROPAGATION FOR LEATHERLEAF FERN (Rumohra adiantiformis) ON THE HIGHLAND OF THAILAND

INTRODUCTION

Fern is an ornamental crop widely grown in gardens, as pot plants and for cut-leaf. In Thailand, fern has been grown since King Rama V (1). It has been grown and propagated commercially since then. There are several methods of fern propagation. For the asexual propagation, clump division, rhizome cutting, but cutting or plantlet cutting are employed. For the sexual propagation, spore culture seems the best method as it could produce large number of plants at a time. Several countries such as the Netherlands used it for the commercial scale production.

Leatherleaf fern, Rumohra adiantiformis (G. Forst) Ching, is a cut-leaf crop. It is grown on a large commercial scale in Florida, the recent total annual value was close to 135 million baht. It is also grown commercially in Central and South Americas in the areas that are free from freezing temperatures. The fronds from this fern have been distributed in the United States, Europe and Japan for flower arrangement (3).

The leatherleaf fern is therefore commercially important in the world market. The work from several experiments indicated that the leatherleaf fern could grow well, with a potential for commercial production in Thailand, especially at the altitude of 1,200 meters above sea level (4). However, to produce leatherleaf fern commercially, it is necessary to study possible methods of its propagation to save the stock import cost which is rather expensive. This study was therefore conducted with the objectives to find the basic inputs in spore culture for commercial propagation application.

MATERIALS AND METHODS

The leatherleaf fern used in the study has been grown for eight years at ground-bed condition containing the forest top soil and leaf mold, of equal volume. The bed was 70 x 150 cm. with cement block edge. The space between plants was 20 cm.; and between rows, 40 cm. The fertilizer baifolan, 14-14-14 (N-P-K) formula, was sprayed over leaves (more dilute than the dose prescribed for general flowers and ornamental plants) every two weeks. Special watering was given during the dry season, from February to June only.

The result of the study was recorded beginning from the sorus development and the maturity of sporangia, the relationship between the size of fertile frond and number of sporangia, and spore collection, techniques of spore culture at the commercial scale, appropriate medium for spore culture, germination, growth and

development of sporophyte, division of plantlets, and the study on the growth rate of leatherleaf fern after division including problems as incurred. (The stud, was financially supported by the USDA in the Developmental Research on Economic Ferns as Cash Crops for the Hilltribes of Northern Thailand during 1978-1981, and the Royal Project and the Ministry of Science, Technology and Energy from 1982 to present). The study site was Doi Pui Research Station, Chiang Mai, elevation 1,200 m.

RESULTS AND DISCUSSION

Sorus development and maturity of sporangia

The leatherleaf fern, after one year of spore culture or stock obtained from rhizome cutting, grew rapidly from May onward. New leaves or non-fertile frond continued to grow till the end of rainy season in October. After this the fertile fronds came out, and, from January to early May, were mature enough to be cut for further propagation.

The relationship between the fertile frond and number of sporangia

By classifying the sizes of fertile frond and the number of the sporangia produced during January to June when the leatherleaf fern produced spore, it was found that the size of fertile frond corresponded to the number of sporangia developed. The details are to be presented later on.

Spore incubation and collection

Leatherleaf fern grown at the altitude of 1,200 m. at Doi Pui Experiment Station developed the sporangia behind the leaf for the period of 3-4 months. The humidity in the air and the soil were the major influence. It was found that the spore matured sooner or the development period of sporangia was shorter if the temperature was high and the humidity in the soil and air were low. This was an observation only; no detailed study was conducted. The mature sporangia ready to be cultured would change color from black to tan. When it was fully mature, the frond were cut and placed up side down on the paper in the transparent bag with open end and kept in the transparentroofed house. Within one week all the spore could be brought out of the sporangia.

Some techniques of the fern spore culture on a commercial scale

Forceps are used to pick off impurities from the spore, such as pieces of fern leaf, inducium or others. It is not necessary to decontaminate microorganisms that come with the spore. More important is the sterilization of the culture dish by boiling, and the medium which must be steamed.

Experiment on leatherleaf fern spore culture in the paddysoil in comparison with chopped Platycerium coronarium

The leatherleaf fern spore was cultured in the medium made of paddy soil (from the Chiang Mai plain) comparing with that of chopped Platycerium coronarium. The result showed that the chopped Platycerium coronarium served best for spore culture. The spore grew to be protallus within 20 days and to sporophyte giving the first frond within 40 days. With the field soil medium, ground and pressed till the surface turned smooth then sterilized by steam, it required 120-150 days for the prothallus to develop and another 30 days for the development of sporophyte.

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EFFECT OF THE FUNGICIDE APPLICATION ON THE PERCENTAGE OF FUNGAL CONTAMINATION AND CONDITION OF LEATHERLEAF FERN SEEDLINGS

INTRODUCTION

Leatherleaf fern was grown mainly as a cut-leaf crop. In Florida, U.S.A., the annual trade value was about 50 million dollars (Nell et al., 1983). In Thailand besides rhizome division, the leatherleaf fern was propagated by sporing, cutting adn tissue culture techniques, on which the Highland Agriculture Project and the Royal Project with the research grants from the USDA had issued reports since 1981 (Highland Agriculture Project, 1981).

Even though the tissue culture technique could produce a great number of stocks within a short time, there were some problems while transferring the plantlets from the test tube or flask so that they could adapt themselves to the natural environment or hardening, or while transferring from the bottle to change the nutrient or medium so that they could develop into sporophyte plants before transplanting into a pot. This study was therefore conducted to understand the effect of the fungicide application on the percentage of fungal contamination and general condition of fern plantlets to be used for the improvement of the leatherleaf fern culture.

MATERIALS AND METHODS

The young plantlets of featherleaf fern during the pre-sporophyte stage, grown from the tissue culture by the Bangkok Flower Center Co., Ltd, were moved from the bottle and washed by the tap water to clear away the agar medium then packed in the moist paper to maintain the humidity. Those were taken to Doi Pui Experiment Station, Chiang Mai on May 18, 1983, when the medium was prepared by using chopped Platycerium coronarium sterilized with 100°C water for one hour then filled in sterile transparent plastic box, 30 x 45 x 22 cm., to the level of approx. 3 cm., and left to cool. The forceps were employed in transplanting. Each box should contain equal number of plantet groups. The chemical treatment employed Vitavax which had the quality of systemic fungicide containing active ingredients -- 75% Carbonin (5, 6-dihydro-2-methyl-1, 4-oxathiin-3-carbonanilide) at 10 gram/litre clean water; the control treatment employed only sterilized water. The experiment was conducted by randomized block design, 3 blocks for each treatment with 4 duplicates. The data were collected on June 4, 1986.

RESULTS AND DISCUSSION

The result shows that spraying the fungicide having the commercial name "Vitavax" once at the rate of 10 grams per 20 litres of clean water could stop the growth of the fungi "saphrophite" which grew from the nutrient agar remaining after washing. For the treatments not using the fungicide, the rate of contamination was 43.3-70.0% or 60.4% average (Table 1).

From the judgement made by scoring of the growth and development of the leatherleaf fern plantlets on the sterized chopped Platycerium coronarium, it was found that the treatment with the fungicide "Vitavax" had higher average score than the treatment without the fungicide. Some groups of the leather-leaf fern not receiving the fungicide changed the color from green to brown and was bruised probably because the fungi though not directly attaching the fern might release certain chemicals and heat which were dangerous to the fern at the pre-sporophyte stage. When put into practice, it might be necessary to spray the fungicide more than once so that it could effectively limit the growth of the fungi poisonous to the fern.

From the study of the percentage of fungal contamination, it was found that the agar nutrient coming with the rhizoid of the leatherleaf fern plantets was important carrier for the epidermic of various kinds of saphrophyte fungi. Therefore it was necessary to completely wash away the agar nutrient by cleaning it in the sieve with clean water for many times and immersing it in the fungicide before planting in the box. This should be a good measure.

The final check was made on July 11, 1986 to find that during the past one month the leatherleaf fern plantlets developed from the pre-sporophyte stage to the period before the frond sprouting thin complete sporophyte with fronds within 2 months (Figure 1).

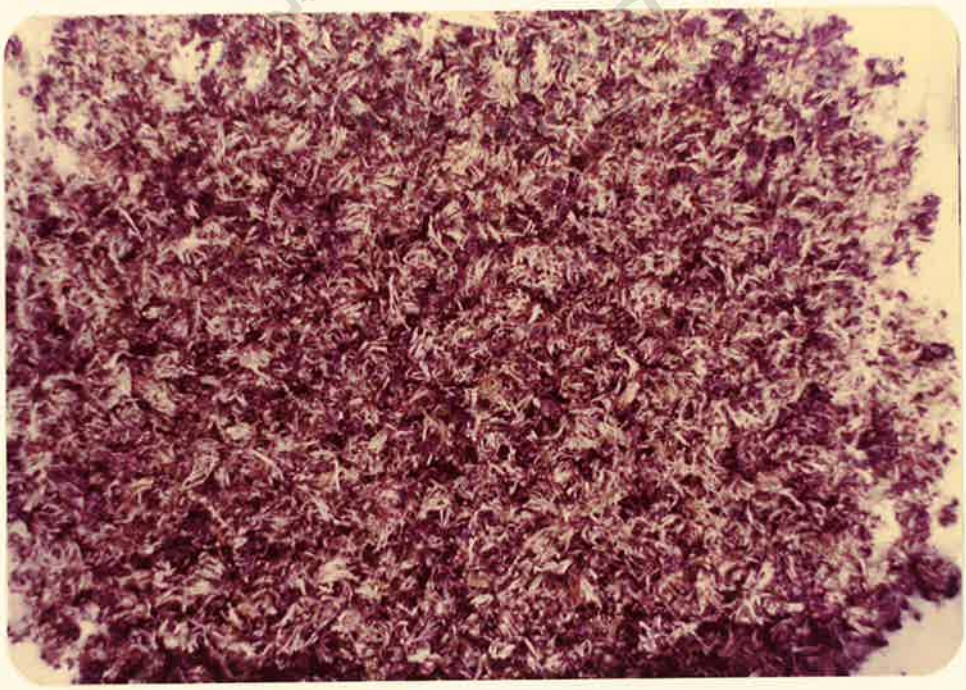

Figure 1. Leatherleaf fern plantlets obtained from tissue culture before planting.

Table 1. Growth and development of leatherleaf fern plantlets.

Treatment	Percentage of fungi					Score of growth				
	Replicate				Average	Replicate				Average
	I	II	III	IV		I	II	III	IV	
Fungicide[1/]	23.3	13.3	14.0	36.6	21.8	4.0	4.0	4.5	3.5	4.0
Without fungicide	43.3	65.0	63.3	70.0	60.4	3.5	3.0	3.0	1.0	2.6

1/ Vitavax : Carbonin 5, 6-dihydro-2methyl-1, 4-oxathiin-3-carbonanilide) 75%.

2/ Score of leatherleaf fern development from greeness and freshness of the plant including growth change, 5 was the highest growth, 1 was the lowest.

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EFFECT OF GROWING MEDIA ON THE RATE OF SURVIVAL AND DEVELOPMENT OF LEATHERLEAF FERN AT THE PRE-SPOROPHYTE STAGE

INTRODUCTION

The leatherleaf fern (Rumohra adiantiformis) is an important cut-leaf crop for many countries, especially Florida of U.S.A., Costa Rica and some countries in the North and South Americas. Leatherleaf fern was introduced into Thailand in 1978 by the Royal Project and could grow well. The leaves are sold fresh for flower arrangements and in the form of dry-preserved. In an effort to propagate this kind of fern, the tissue culture techniques have been conducted and rhizomes were imported by the Bangkok Flower Center Co., Ltd. for commercial cultivation in Kanchanaburi. However, the tissue culture practice was not so successful since some of the fern plantlets as obtained have not developed to the sporophyte stage. Therefore, a study has been conducted to find growing media suitable for the fern plantlets during the pre-sporophyte stage for further commercial cultivation.

MATERIALS AND METHODS

The experiment was started on July 26, 1986 at the Doi Pui Experiment Station, Chiang Mai province. The randomized block design was employed for the 6 treatments involving various kinds of growing media commonly available in Chiang Mai as follows :

Treatment 1 - fresh sphagnum moss

Treatment 2 - finely-chopped coconut fibre

Treatment 3 - saw dust

Treatment 4 - leaf mold

Treatment 5 - saw dust and coarse sand, 1:1

Treatment 6 - topsoil

Each treatment was done in triplicate; 300 g. of tissue-cultured leather leaf fern plantlets were employed in each replicate.

Three experimental plots were set up in the shady area (75% shade); the surface soil was finely graded and elevated to 6 inches above ground level. Each plot was divided into six equal parts (shown in diagram), sprayed with Dieldrin to prevent the white ants and ants. Then 1-inch thick of prepared growing media were put on the surface soil and the fungicide Vitavax was applied.

	TREATMENTS					
R_1	6	3	4	2	5	1
R_2	2	4	5	3	1	6
R_3	5	3	1	6	2	4

Experimental plot diagram.

The fern plantlets were weighed and divided, 50 g. of sloutlets for each treatment. The plantlets were spread equally on each sub-plot and watered with mist shower. A plastic roof was used for covering the plots in the tunnellike form to maintain constant humidity. The fungicide was sprayed every two weeks and water was given each week. The data on the survival rate were recorded 30 days after transplanting, August 25, 1986. The development into the frond was scored 60 days after transplanting, September 23, 1986.

RESULTS

From the experiment on various kinds of media, the leaf mold of Quercus spp. was the best growing medium for leatherleaf fern plantlets. The plantlets survived on them more than any other media. The reasons might be the looseness and water absorptability and mild acidity including nutrients which were appropriate for the

fern plantlets. The disadvantage was that it degenerated rapidly if used in rather high temperature environment. The second best was the finely-chopped coconut fibre and topsoil. The rate of plantlet survival was very near to the first one. The sawdust and the mixture of sawdust and coarse sand were not quite effective which might be due to the lack of principal nutrients required by the fern plantlets. Sphagnum moss gave the lowest rate of survival -- 7.7 per cent and the score of frond development was rather low mainly because of the physical condition which allowed for large amount of water absorption. Moreover, the sphagnum moss died and decayed very quickly leading to the infestation of bacteria and fungi detrimental to the fern plantlets.

For the frond development, all kinds of growing media could make the frond sprout. However, the use of the leaf male and topsoil as the growing media resulted with consistently larger frond than any other growing media. The second best was the finely-chopped coconut fibre.

CONCLUSION

It could be summarized that in nursing the leatherleaf fern planlets before the frond sprouting stage, we could use the leaf mold of Quercus spp. which was generally available in the forests. Those leaf mold when left to decompose made the most effective growing media at a low cost. The leatherleaf fern plantlets grown on such medium for 3-5 months could be separated to grow in a single pot.

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