Chapter 4

Results

Part I Studies on factors influencing growth and *in vitro* tuberization.

Experiment 1 Effect of sucrose concentration

Effects of various sucrose concentrations were evaluated in terms of vegetative growth, tuberization and root growth.

1.1 Vegetative growth

After the protocorms were transferred onto the CMU_1 medium varying in sucrose concentrations with increasing light intensity, the protocorm-derived plantlets developed chlorophyll pigment in their young leaves since they were inside their leaf sheaths. After that, the young leaves slowly emerged and leaf blades expanded in the first week after culturing, and increased in size in later weeks. Plantlets cultured in the CMU_1 medium without sucrose or with 1 % sucrose gave rapid leaf emergence and more leaf emerging percentages than those cultured in very high concentrations of sucrose, i.e. 5 and 7 % (Table 4).

Table 4 Effect of sucrose concentration on leaf-emerging percentage of *Pecteilis sagarikii* seedlings during culturing for 10 weeks.

Sucrose (%)	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
0	30	80	95	100	100	100	100	100	100	100
1	35	80	90	100	100	100	100	100	100	100
2	10	40	75	95	100	100	100	100	100	100
3	15	15	90	100	100	100	100	100	100	100
5	15	30	65	80	85	85	95	100	100	100
7	5	10	15	45	60	70	85	95	95	100

After culturing for 10 weeks, the sucrose concentrations have no significant effect on shoot height and number of leaf per plant, but gave significant effect on leaf width and leaf length. However, adding sucrose at 0 to 5 % showed no significant difference in leaf width, but gave significantly wider leaf than that from the 7 % concentration. Adding sucrose at 0 and 1 % gave more leaf length than those cultured in higher sucrose concentration medium. The leaf grew well and produced the biggest size when cultured in the CMU_1 medium with sucrose at 1 % or without sucrose. Leaf length reduced markedly in the 2 – 5 % sucrose levels, and stunted in the 7 % sucrose level (Table 5).

Table 5 Effect of sucrose concentration on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Sucrose (%)	Shoot height (mm)	No. of leaf / plant	Leaf width (mm)	Leaf length (mm)
0	3.4±0.6	1.7±0.5	4.1±1.4 a	7.9±2.0 ab
1	3.2±0.5	1.6±0.6	4.1±1.1 a	9.0±3.4 a
2	3.4±0.5	1.6±0.5	3.7±1.2 a	6.8±2.1 bc
3	3.0±0.6	1.7±0.5	3.3±1.2 a	6.0±2.8 c
5	3.3±0.4	1.5±0.5	3.3±1.5 a	5.6±3.0 c
7	3.2±0.5	1.5±0.5	2.0±1.2 b	3.2±1.7 d
$LSD_{.05}$	*NS*	*NS*	*0.8*	*1.6*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

1.2 Tuberization and growth

The sign of *in vitro* tuberization could be observed from an external change. A tuber primordium was observed to emerge at the base of a plantlet in the opposite direction of its protocorm. The forming position was between the shoot and the protocorm body, and then extended straight down into the medium. After the initial protocorms were transferred onto the tested media, plantlets cultured in the CMU_1 medium adding 3 % sucrose began to form tubers in the first week, but in the other media tuberization occurred in the second week after culturing, and the percentage of tuberization increased in later weeks. After culturing for 10 weeks, the plantlets cultured in the medium without sucrose or with sucrose up to 5 % generally gave better tuberization rate and more tuberization percentages than those cultured in the medium adding sucrose at the highest concentration at 7 % (Table 6).

Table 6 Effect of sucrose concentration on tuberization percentage of *Pecteilis sagarikii* seedlings during culturing for 10 weeks.

Sucrose (%)	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
0	0	20	30	50	70	70	75	90	90	90
1	0	15	35	80	85	90	90	90	95	95
2	0	15	30	50	65	75	80	80	80	80
3	10	20	20	50	65	85	90	95	95	95
5	0	15	50	60	70	75	80	80	90	90
7	0	10	15	35	35	50	55	60	70	75

For tuber growth, it was found that after the tuber primordium emerged, it continued to grow straight down into the medium to produce a tuber stalk by increasing in its length rather than its width. After culturing for 7 weeks, the tuber that had an appropriate length began to markedly increase its width. The increase in

tuber width could be found in 2 patterns resulting in different tuber shapes, i.e. 1) oval-shape tuber, derived from normal extension of tuber stalk with particular oval expansion of a tuber body under the tuber shoot bud resembling the tuber formation in nature, and 2) cylindrical-shape tuber, derived from an equal expansion throughout a part of the tuber stalk and tuber tip, or only short tuber stalk extension (Figure 2).

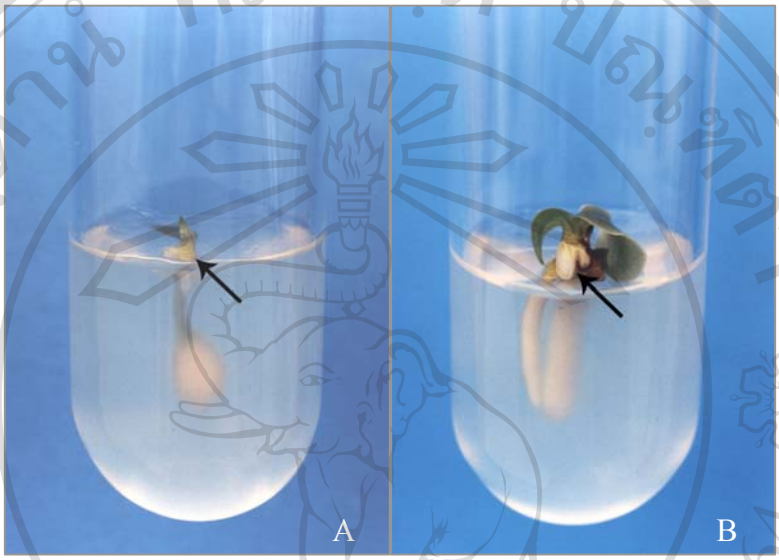


Figure 2 *Pecteilis sagarikii* seedlings showing two different tuber shapes and the new tuber primordia (arrow).
A) oval-shape tuber
B) cylindrical-shape tuber

After culturing for 9 weeks, some plantlets produced new tuber primordia. They emerged in the position close to the first tuber appearance, probably occurred directly at the same place as the first tuber by passing through the upper part of tuber stalk to split open. Thereafter, the new tuber primordia developed into meristematic tissue that appeared to be a protocorm-like body and then subsequently produced its tuber (Figure 2).

After culturing for 10 weeks, the plantlets cultured in the media of different sucrose concentrations were not significantly different in producing number of tuber per plant, at an average of 0.8±0.5 to 1.1±0.6 tubers/plant. However, the sucrose concentrations had significant effect on tuber size. An increase in sucrose concentration decreased tuber length more than tuber width. Plantlets cultured on the media adding sucrose concentration at 0 to 5% provided no significantly different in tuber width, but gave bigger width than those cultured in 7 %; whereas tuber length was significantly decreased when cultured in the media adding sucrose at 2 to 5 %, the shortest one was obtained from the highest concentration at 7 %. Tubers grew well and produced the biggest size when cultured in the medium adding 1 % sucrose (Table 7 and Figure 3).

For the tuber shape, it was found that plantlets cultured in the tested media having sucrose adding at 0 to 5 % produced the oval-shape tuber at 15 to 25 %, while those from the medium adding 7 % sucrose produced only 10 % (Table 7).

Table 7 Effect of sucrose concentration on number of tuber, tuber width and tuber length, and oval-shape tuber percentage of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Sucrose (%)	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)	Oval-shape tuber (%)
0	1.1±0.6	2.2±1.3 a	10.3±5.9 ab	20
1	1.0±0.3	2.7±1.3 a	12.5±6.3 a	20
2	1.0±0.7	2.0±1.3 ab	8.0±5.5 b	15
3	1.0±0.3	2.2±1.1 a	8.3±4.4 b	15
5	0.9±0.3	2.3±1.2 a	7.3±4.6 b	25
7	0.8±0.5	1.3±1.2 b	3.4±4.0 c	10
$LSD_{.05}$	*NS*	*0.8*	*3.2*	*NA*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different, NA= not statistical analysed

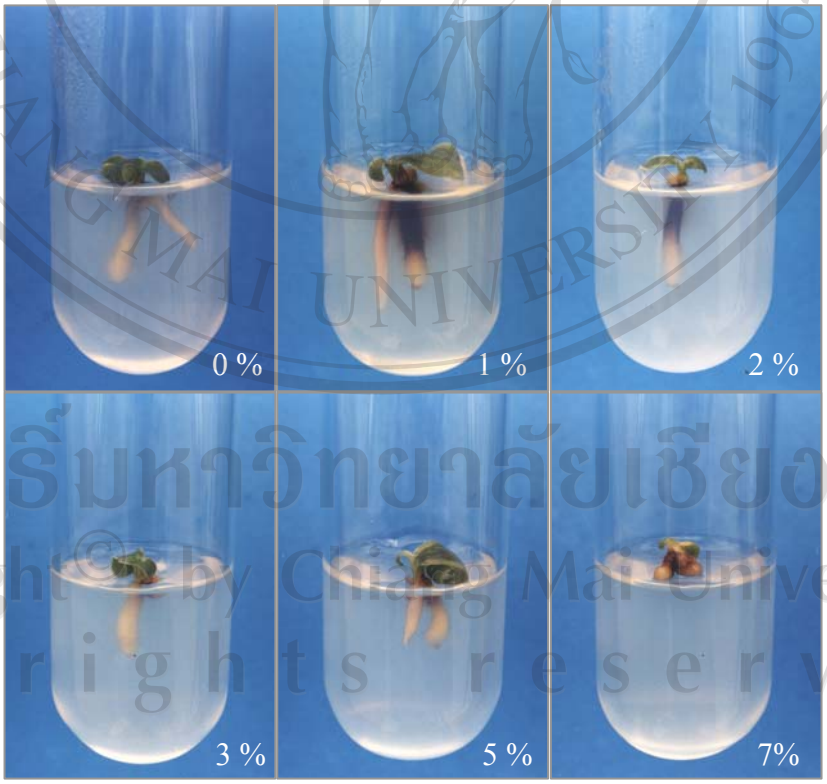


Figure 3 *Pecteilis sagarikii* seedlings cultured in CMU_1 medium with different sucrose concentrations at 0 1 2 3 5 and 7 % (w/v) for 10 weeks.

1.3 Root formation and growth

Plant cultured in the medium adding sucrose at 3 % rooted after culturing for 1 week, but in the other media they produced in the second week, excepted in the medium adding sucrose at the highest concentration at 7 %, root occurred after culturing for 3 weeks. In addition, the highest sucrose concentration not only affected in delaying root formation but also markedly decreased in rooting percentage during culturing for 10 weeks (Table 8). However, adding sucrose concentration at 0 to 7 % had no significant effect on number of root, root width and root length after culturing for 10 weeks, nevertheless the highest concentration tended to decrease root growth in terms of root length (Table 9).

Table 8 Effect of sucrose concentration on rooting percentage of *Pecteilis sagarikii* seedlings during culturing for 10 weeks.

Sucrose (%)	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
0	0	25	25	35	40	40	40	40	40	45
1	0	30	40	50	50	50	50	50	50	50
2	0	25	45	45	55	55	55	55	55	55
3	5	25	30	40	40	40	45	45	45	45
5	0	20	30	45	50	50	50	50	50	55
7	0	0	10	20	20	20	20	20	20	30

Table 9 Effect of sucrose concentration on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Sucrose (%)	No. of root / plant	Root width (mm)	Root length (mm)
0	0.5±0.5	0.5±0.6	3.5±4.7
1	0.6±0.6	0.6±0.6	4.7±5.6
2	0.6±0.5	0.7±0.7	4.6±5.8
3	0.5±0.5	0.6±0.8	4.2±6.8
5	0.6±0.5	0.6±0.6	3.7±4.5
7	0.3±0.5	0.3±0.5	0.5±0.9
$LSD_{.05}$	*NS*	*NS*	*NS*

NS= not significantly different

Experiment 2 Effect of coconut water concentration

Effects of various coconut water concentrations were investigated and evaluated in terms of vegetative growth, tuberization and root growth.

2.1 Vegetative growth

After the protocorms were transferred onto the CMU_1 medium having coconut water at different concentrations and illuminated similar to the experiment 1, the developing plantlets started to develop chlorophyll pigment in their young leaves before emerging from leaf sheath, and then expanded to functional leaf, similar to that occurred in the experiment 1. Plantlets cultured in the CMU_1 medium without coconut water produced the lowest leaf emerging percentage in the first week. However, those plantlets cultured in 0 – 15 % coconut water media yielded almost the same leaf emerging percentages since the second week after culturing, but when adding coconut water at the highest concentration the percentage tended to be lower (Table 10).

Table 10 Effect of coconut water concentration on leaf-emerging percentage of *Pecteilis sagarikii* seedlings during culturing for 10 weeks.

Coconut water (%)	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
0	10	65	85	100	100	100	100	100	100	100
5	40	65	100	100	100	100	100	100	100	100
10	50	75	90	95	95	100	100	100	100	100
15	40	60	90	95	95	100	100	100	100	100
20	25	55	95	95	95	95	100	100	100	100

After culturing for 10 weeks, plantlets cultured in the medium containing different concentrations of coconut water or without it gave no significant difference in shoot height, number of leaf per plant, leaf width, and also leaf length (Table 11).

Table 11 Effect of coconut water concentration on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Coconut water (%)	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
0	3.0±0.5	1.7±0.7	3.2±0.8	7.1±2.4
5	3.2±0.5	1.6±0.5	3.5±1.3	7.3±2.3
10	3.2±0.4	1.3±0.5	4.0±1.3	8.4±3.3
15	3.2±0.6	1.7±0.7	3.5±0.9	7.4±2.3
20	3.3±0.5	1.4±0.6	3.9±1.3	8.2±3.1
$LSD_{.05}$	*NS*	*NS*	*NS*	*NS*

NS= not significantly different

2.2 Tuberization and growth

Plantlets cultured in the CMU_1 medium adding coconut water at 0, 5, 10 and 15 % (v/v) formed tuber primordia which could be visually observed in the first week after culturing, nevertheless in the highest coconut water concentration at 20 % they produced tuber primordia after culturing for 2 weeks. The cultured plantlets in

all treatments showed the highest tuberization percentages at 90 to 100 % after culturing for 8 weeks (Table 12).

Table 12 Effect of coconut water concentration on tuberization percentage of seedlings during culturing for 10 weeks.

Coconut water (%)	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
0	10	10	20	50	65	80	100	100	100	100
5	10	20	30	60	90	95	95	95	95	95
10	10	15	20	65	85	85	95	100	100	100
15	5	20	25	60	65	85	90	95	95	95
20	0	5	40	65	80	85	90	90	90	90

For tuber growth, it was found that after tuber primordia emerged and continued to grow straight down into the medium, plantlets cultured in the medium containing coconut water at 10 to 20 % started to enlarge their tuber width after culturing for 6 weeks, faster than those cultured in the medium having coconut water at 5 % which appeared in the week seventh. The latest tuber enlargement was found in the medium without coconut water, 9 weeks after culturing.

In addition, some plantlets produced new tuber primordia that developed into protocorm-like bodies after culturing for 9 weeks, in the same manner as found in the first experiment.

After culturing for 10 weeks, it showed that coconut water at different concentrations had no significant effect on the number of tuber per plant and tuber length, but differently affected tuber width. Plantlets cultured in the media with coconut water at 5 to 20 % produced more tuber width than those obtained from the medium without coconut water (Table 13 and Figure 4).

For the tuber shape, it showed that plantlets cultured in the medium having coconut water yielded more oval-shape tuber than those in the medium without coconut water which produced only 15 % (Table 13).

Table 13 Effect of coconut water concentration on number of tuber, tuber size and oval-shape tuber percentage of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Coconut water (%)	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)	Oval-shape tuber (%)
0	1.2±0.5	1.4±0.8 b	8.8±4.1	15
5	1.0±0.2	2.1±0.8 ab	13.4±5.4	20
10	1.0±0	2.5±1.2 a	12.3±5.7	25
15	1.0±0.2	2.5±1.7 a	10.1±7.0	30
20	0.9±0.3	2.4±1.5 a	9.8±6.6	20
$LSD_{.05}$	*NS*	*0.78*	*NS*	*NA*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different, NA= not statistical analysed

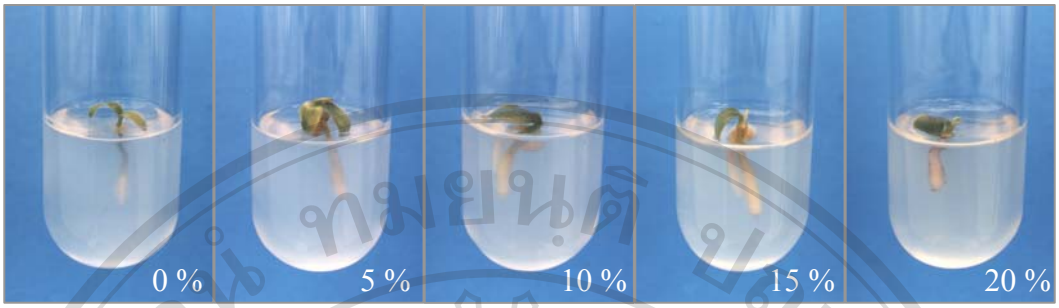


Figure 4 *Pecteilis sagarikii* seedlings cultured in CMU_1 medium with different coconut water concentrations after culturing for 10 weeks.

2.3 Root formation and growth

During culturing for 10 weeks, plantlets cultured in the media containing coconut water at 15 and 20 % produced root in the first week, whereas in the other media root appeared in the second week after culturing. However, all coconut water treatments have oscillated effect on rooting percentage after culturing for 10 weeks (Table 14). In addition, adding coconut water at the tested concentrations gave no pronounced effect on number of root, root width and root length after culturing for 10 weeks (Table 15).

Table 14 Effect of coconut water concentration on rooting percentage of seedlings during culturing for 10 weeks.

Coconut water (%)	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
0	0	5	30	40	40	45	45	45	45	45
5	0	15	30	30	30	30	30	30	30	30
10	0	35	40	45	45	45	45	45	45	45
15	10	30	45	55	55	55	55	55	55	55
20	5	15	30	35	40	45	45	45	45	50

Table 15 Effect of coconut water concentration on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Coconut water (%)	No. of root / plant	Root width (mm)	Root length (mm)
0	0.5±0.5	0.5±0.5	3.1±3.8
5	0.3±0.5	0.3±0.5	2.5±4.0
10	0.5±0.5	0.5±0.6	4.5±5.9
15	0.6±0.5	0.7±0.7	5.5±6.8
20	0.5±0.5	0.6±0.7	3.2±4.2
$LSD_{.05}$	*NS*	*NS*	*NS*

NS= not significantly different

Experiment 3 Effects of coconut water and BAP concentrations

This experiment was carried out to find the effects of coconut water and BAP concentrations and their interaction on vegetative growth, tuberization and root growth.

3.1 Vegetative growth

Plantlets cultured in the CMU_1 medium varying in coconut water and BAP concentrations showed morphological changes at the same time as occurred in the experiments 1 and 2, i.e. developing of chlorophyll pigment in young leaf, followed by leaf emerging and leaf expansion. However, increasing BAP concentration affected in delaying leaf emergence; but adding coconut water seemed to reduce BAP effectiveness (Table 16).

Table 16 Effects of coconut water and BAP concentrations on leaf-emerging percentages of the protocorms during culturing for 10 weeks.

CW	BAP	Time after culturing (weeks)									
(%)	(mg/l)	1	2	3	4	5	6	7	8	9	10
0	0	30	90	90	100	100	100	100	100	100	100
	0.1	10	40	70	90	100	100	100	100	100	100
	0.5	10	30	50	90	90	90	90	90	90	100
	1.0	0	30	70	80	80	80	80	80	100	100
	2.0	0	10	30	50	60	60	60	60	60	100
15	0	10	90	90	90	100	100	100	100	100	100
	0.1	0	60	60	70	100	100	100	100	100	100
	0.5	10	60	70	70	100	100	100	100	100	100
	1.0	10	60	60	60	60	70	90	90	90	90
	2.0	0	20	50	60	60	60	70	90	90	100

Main effect of coconut water

Coconut water at 15 % gave better growth in terms of shoot height, but it had no significant effect on number of leaf per plant. Adding coconut water had, nevertheless, pronounced effect on leaf width and leaf length (Table 17).

Table 17 Main effect of coconut water concentration on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Coconut water (%)	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
0	3.4±0.7 b	1.4±0.5	1.9±1.2 b	3.9±2.1 b
15	3.9±1.3 a	1.5±0.6	3.0±1.7 a	5.5±2.8 a
$LSD_{.05}$	*0.36*	*NS*	*0.52*	*0.88*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Main effect of BAP

BAP also gave significant better growth in terms of shoot height, especially at higher concentrations from 0.5 – 2.0 mg/l. But it had no significant effect on number of leaf per plant. In contrast, increasing BAP especially at higher concentration than 0.5 mg/l has significant negative effect on leaf width and leaf length. Plantlets gave the biggest leaf size when cultured in the medium having BAP at low concentration at 0.1 mg/l or in the absence of BAP (Table 18).

Table 18 Main effect of BAP concentration on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

BAP (mg/l)	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
0	2.8±0.6 d	1.6 ± 0.6	3.1±1.4 a	6.5±2.8 a
0.1	3.4±0.7 c	1.6 ± 0.5	3.1±1.5 a	5.4±2.5 ab
0.5	4.3±1.4 a	1.5 ± 0.5	2.7±1.6 ab	4.8±2.1 bc
1.0	3.7±0.8 bc	1.2 ± 0.5	1.9±1.4 bc	3.9±2.3 cd
2.0	4.2±1.1 ab	1.4 ± 0.6	1.4±1.1 c	2.8±1.7 d
$LSD_{.05}$	*0.57*	*NS*	*0.82*	*1.39*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Interaction effects of coconut water and BAP

Plantlets cultured in the CMU_1 medium supplied with different concentrations of coconut water and BAP gave different results in terms of shoot height and leaf size, but not significantly affected number of leaf per plant. In addition, adding BAP at 0.1 to 2.0 mg/l in the medium without coconut water promoted plant height which was more pronounced in the medium with coconut water and also BAP at 0.5 to 2.0 mg/l. In contrast, increasing BAP especially at the highest concentration at 2.0 mg/l resulted in decreasing leaf size. However, adding coconut water at 15 % with BAP at different concentrations gave more leaf size than adding only BAP. After culturing for 10 weeks, the plantlets cultured in the media supplemented with coconut water at 15 % together with BAP at 0, 0.1 and 0.5 mg/l gave the most leaf width and leaf length (Table 19).

Table 19 Effects of coconut water and BAP concentrations on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

CW (%)	BAP (mg/l)	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
0	0	2.6±0.3 d	1.6 ± 0.7	2.4±0.6 b	5.7±1.4 abc
	0.1	3.5±0.6 bc	1.5 ± 0.5	2.4±1.1 b	4.6±2.1 bcd
	0.5	3.5±0.6 bc	1.4 ± 0.5	1.8±1.2 bc	3.8±1.7 cde
	1.0	3.6±0.8 bc	1.2 ± 0.4	1.7±1.4 bc	3.2±2.0 de
	2.0	3.8±0.9 b	1.2 ± 0.4	1.1±1.0 c	2.1±1.3 e
15	0	2.9±0.8 cd	1.5 ± 0.5	3.7±1.7 a	7.3±3.6 a
	0.1	3.4±0.8 bcd	1.6 ± 0.5	3.7±1.7 a	6.3±2.7 ab
	0.5	5.1±1.5 a	1.6 ± 0.5	3.6±1.5 a	5.8±2.1 ab
	1.0	3.8±0.9 b	1.2 ± 0.6	2.2±1.5 bc	4.5±2.4 bcd
	2.0	4.6±1.3 a	1.5 ± 0.7	1.8±1.1 bc	3.5±1.7 de
	$LSD_{.05}$	*0.8*	*NS*	*1.17*	*1.96*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

3.2 Tuberization and growth

Plantlets cultured in the CMU_1 medium varying in coconut water and BAP concentrations started tuberization after culturing for 1 week. During culturing for 10 weeks, it showed that increasing BAP concentration had negative effect on tuberization percentage and also delaying in tuber formation. However, adding coconut water gave more tuberization percentage than those cultured in the same levels of BAP concentration, but without coconut water (Table 20).

Table 20 Effects of coconut water and BAP concentrations on tuberization percentages of *Pecteilis sagarikii* seedlings during culturing for 10 weeks.

CW	BAP	Time after culturing (weeks)									
(%)	(mg/l)	1	2	3	4	5	6	7	8	9	10
0	0	30	40	60	80	100	100	100	100	100	100
	0.1	10	30	40	40	40	70	80	80	80	80
	0.5	0	10	20	20	20	20	20	20	20	30
	1.0	0	10	10	20	20	20	30	30	30	30
	2.0	10	10	10	10	10	10	10	10	20	20
15	0	20	40	40	80	100	100	100	100	100	100
	0.1	10	40	60	60	90	100	100	100	100	100
	0.5	10	10	40	60	60	70	80	80	80	80
	1.0	10	20	30	30	30	30	30	40	40	50
	2.0	0	10	10	10	10	40	40	40	40	40

Main effect of coconut water

Adding coconut water at 15 % had no significant effect in promoting number of tuber per plant. However, coconut water significantly promoted growth in terms of tuber width and tuber length (Table 21).

Table 21 Main effect of coconut water concentration on number of tuber and tuber size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Coconut water (%)	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)
0	0.7±0.8	1.3±1.6 b	4.6±5.6 b
15	0.8±0.5	3.9±3.2 a	9.2±7.1 a
$LSD_{.05}$	*NS*	*0.96*	*1.65*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Main effect of BAP

BAP had significant effect on number of tuber per plant, tuber width and also tuber length. But, increasing BAP had an adverse effect, to decrease number of tuber per plant. Adding BAP at 0.1 and 0.5 mg/l or in its absence gave wider tuber than those cultured in BAP at higher levels at 1.0 and 2.0 mg/l. Similarly, adding BAP at 0.1 mg/l or without BAP obviously promoted growth in terms of tuber length, while higher concentration at 0.5 to 2.0 mg/l gave distinctly significant decrease in tuber length (Table 22).

Table 22 Main effect of BAP concentration on number of tuber and tuber size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

BAP (mg/l)	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)
0	1.3±0.7 a	2.5±1.2 ab	12.4±3.2 a
0.1	1.0±0.4 b	3.8±2.1 a	12.6±7.0 a
0.5	0.6±0.6 c	3.0±3.3 ab	4.8±5.3 b
1.0	0.4±0.5 c	2.3±3.5 b	2.4±3.8 b
2.0	0.3±0.5 c	1.5±2.9 b	2.2±4.4 b
$LSD_{.05}$	*0.32*	*1.53*	*2.62*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05

Interaction effect of coconut water and BAP

Adding coconut water and BAP at various concentrations had positive interaction effects on number of tuber, tuber width and tuber length. For number of tuber per plant, increasing BAP concentration resulted in less producing tuber per plant both in the absence and presence of coconut water. Furthermore, coconut water, without BAP, significantly affected tuber production, but significantly gave better

result when having BAP at 0.5 mg/l than those produced in only the BAP medium. The plantlets produced the highest number of tuber when cultured in the medium without both coconut water and BAP. For tuber width, adding BAP at 0.1 mg/l in combination with coconut water, the highest width was obtained, but tended to decrease when having BAP at higher level. Increasing BAP concentration in coconut water-absent media gave significant decreased tuber length. But, absence BAP or presence BAP at 0.1 mg/l with coconut water significantly increased tuber length. The tubers grew well and produced the biggest size when cultured in the medium adding coconut water at 15 % and BAP at 0.1 mg/l (Table 23 and Figure 5).

For the tuber shape, increasing BAP concentration has resulted to decrease the percentage of oval-shape tuber. In addition, plantlets cultured in the medium without both coconut water and BAP gave 70 % oval-shape tuber, but when was added BAP at 0.1 mg/l, the percentage decreased to 10 %, and, interestingly, no formation of the oval-shape tuber was found when using BAP at higher level at 0.5 to 2.0 mg/l; but when only coconut water was used, plantlets produced lower percentage of the oval-shape tuber at 40 %, and did not produce any oval-shape tuber when cultured onto the medium plus with BAP at 1.0 and 2.0 mg/l. Interestingly, adding coconut water together with BAP at 0.1 – 0.5 mg/l proved better oval-shape tuber formation than having only BAP at the same levels (Table 23).

Table 23 Effects of coconut water and BAP concentrations on number of tuber, tuber size and oval-shape tuber percentage of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

CW (%)	BAP (mg/l)	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)	Oval-shape tuber (%)
0	0	1.6±0.8 a	1.9±0.7 cde	11.3±2.7 bc	70
	0.1	0.9±0.6 bc	2.3±1.5 cde	8.2±6.3 c	10
	0.5	0.3±0.5 d	0.9±1.5 e	1.3±2.2 d	0
	1.0	0.3±0.5 d	1.1±2.1 de	1.5±3.0 d	0
	2.0	0.2±0.4 d	0.5±1.3 e	0.6±1.6 d	0
15	0	1.0±0 b	3.2±1.3 bcd	13.6±3.5 ab	40
	0.1	1.0±0 b	5.4±1.4 a	17.1±4.3 a	20
	0.5	0.9±0.6 bc	5.1±3.4 ab	8.2±5.3 c	10
	1.0	0.5±0.5 cd	3.4±4.4 abc	3.4±4.4 d	0
	2.0	0.4±0.5 d	2.5±3.7 cde	3.8±5.7 d	0
$LSD_{.05}$		*0.45*	*2.16*	*3.70*	*NA*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NA = not statistical analysed

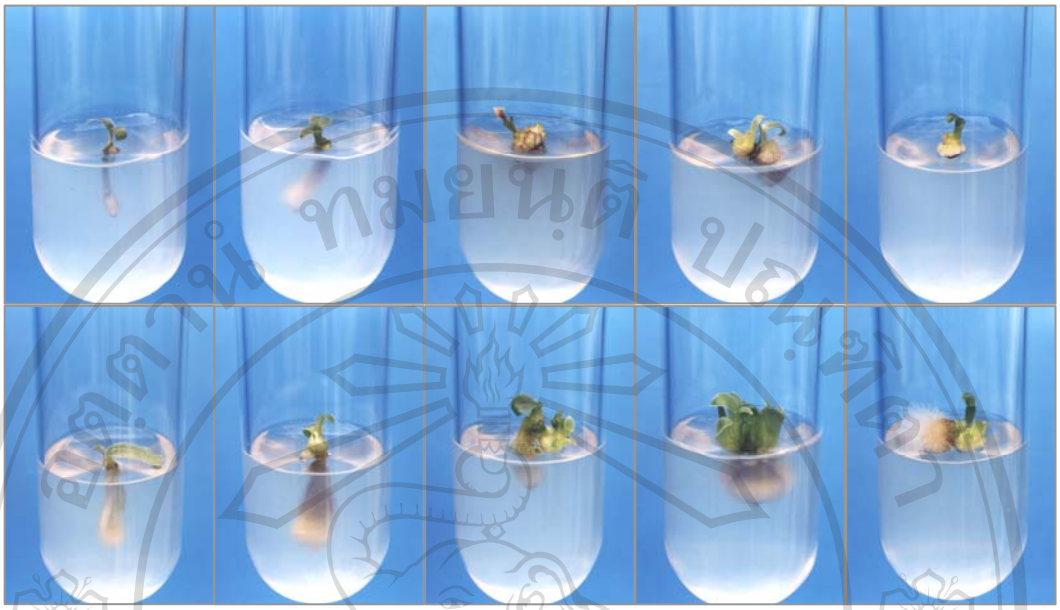


Figure 5 *Pecteilis sagarikii* seedlings cultured in various supplemented CMU_1 medium after culturing for 10 weeks.
A) Without coconut water B) With 15 % coconut water
From left to right: BAP concentration at 0, 0.1, 0.5, 1.0 and 2.0 mg/l, respectively.

3.3 Root formation and growth

After culturing for 10 weeks, increasing BAP concentration showed negative effect to decrease rooting percentage both in the absence and presence of coconut water. Coconut water gave better rooting percentage when used with BAP at 0.1 and 0.5 mg/l. However, plantlets gave highest rooting percentage when cultured in the medium adding only coconut water at 15 % (Table 24).

Table 24 Effects of coconut water and BAP concentrations on rooting percentages of *Pecteilis sagarikii* seedlings during culturing for 10 weeks.

CW	BAP	Time after culturing (weeks)									
(%)	(mg/l)	1	2	3	4	5	6	7	8	9	10
0	0	0	0	0	20	20	40	40	40	40	40
	0.1	0	10	10	10	10	10	10	10	10	20
	0.5	0	0	0	10	10	10	10	10	10	10
	1.0	10	10	10	20	30	30	30	30	30	30
	2.0	0	10	10	20	20	20	20	20	20	20
15	0	20	60	60	70	70	70	70	70	70	70
	0.1	0	40	40	40	40	50	50	50	50	50
	0.5	0	20	20	20	20	30	30	30	30	30
	1.0	0	20	20	30	30	30	30	30	30	30
	2.0	0	10	10	10	10	20	20	20	20	20

Main effect of coconut water

Coconut water had significant effects on increasing root width and root length, but had no significant effect on number of root per plant (Table 25).

Table 25 Main effect of coconut water concentration on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Coconut water (%)	No. of root / plant	Root width (mm)	Root length (mm)
0	0.2±0.4	0.3±0.6 b	1.5±3.0 b
15	0.4±0.5	0.6±0.8 a	4.0±5.3 a
$LSD_{.05}$	*NS*	*0.28*	*1.67*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Main effect of BAP

BAP concentration had no significant effect on number of root per plant, root width and root length, although BAP at higher concentrations showed the overall view in suppressing root length (Table 26).

Table 26 Main effect of BAP concentration on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

BAP (mg/l)	No. of root / plant	Root width (mm)	Root length (mm)
0	0.6±0.5	0.6±0.6	4.5±5.2
0.1	0.4±0.5	0.6±0.8	3.3±5.0
0.5	0.2±0.4	0.4±0.8	1.8±4.0
1.0	0.3±0.5	0.4±0.7	2.6±4.4
2.0	0.2±0.4	0.3±0.6	1.6±3.2
$LSD_{.05}$	*NS*	*NS*	*NS*

NS= not significantly different

Interaction effect of coconut water and BAP

Using coconut water and BAP in combination at various concentrations had no interaction effects on number of root and root width, but gave significant effect in root length. Adding only coconut water or together with 0.1 mg/l BAP gave the best result in root length; but when increasing BAP concentrations, the root length was decreased, similar to rooting in the media without coconut water and adding only BAP at the tested concentrations (Table 27).

Table 27 Effects of coconut water and BAP concentrations on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

CW (%)	BAP (mg/l)	No. of root / plant	Root width (mm)	Root length (mm)
0	0	0.4±0.5	0.3±0.4	1.6±2.8 bc
	0.1	0.2±0.4	0.4±0.7	1.7±3.5 bc
	0.5	0.1±0.3	0.1±0.3	0.4±1.3 c
	1.0	0.3±0.5	0.4±0.7	2.2±4.0 bc
	2.0	0.2±0.4	0.3±0.5	1.6±3.3 bc
15	0	0.7±0.5	1.0±0.7	7.3±5.7 a
	0.1	0.5±0.5	0.8±0.9	4.9±5.9 ab
	0.5	0.3±0.5	0.7±1.1	3.2±5.2 bc
	1.0	0.3±0.5	0.5±0.8	3.0±4.9 bc
	2.0	0.2±0.4	0.4±0.7	1.6±3.3 bc
$LSD_{.05}$		*NS*	*NS*	*3.74*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

3.4 Protocorm-like bodies formation

After the protocorms were transferred onto the tested medium having BAP for 2 weeks, their shoot-base tissues started to be swollen. This morphological change was distinctly observed after culturing for 4 weeks, and then developed to form protocorm-like bodies (plbs) which had shoot primordia at their tips. These plbs initiated at the region between the original shoot and protocorm body. They could form individually or fused together at the original protocorm base (Figure 6).

After culturing for 10 weeks, it was found that increasing BAP concentration and adding coconut water gave an increase in protocorm-like bodies forming percentage, except in the medium adding coconut water with the highest level of BAP at 2.0 mg/l which slightly decreased the plbs formation when compared with the medium having only BAP (Table 28).

At the end of study, the healthy plbs growing in the media adding coconut water with BAP at 0.5 – 1.0 mg/l individually developed theirs shoots and tubers.

Main effect of coconut water

Coconut water had no significant effect on number of plbs per plant (Table 29).

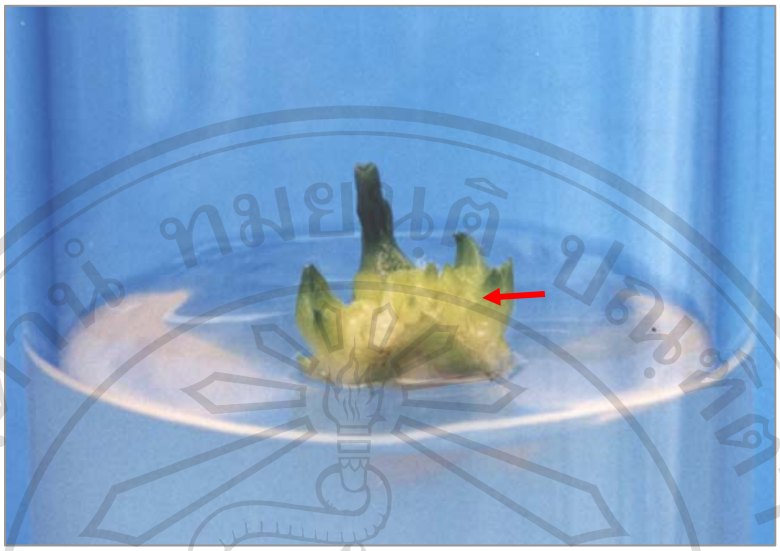


Figure 6 *Pecteilis sagarikii* seedling cultured on CMU_1 medium supplemented with coconut water at 15 % and BAP at 2.0 mg/l produced protocorm-like bodies (arrow) at the joining position between shoot and its protocorm.

Table 28 Effects of coconut water and BAP concentrations on protocorm-like bodies forming percentages of *Pecteilis sagarikii* seedlings during culturing for 10 weeks.

CW	BAP	Time after culturing (weeks)									
(%)	(mg/l)	1	2	3	4	5	6	7	8	9	10
0	0	0	0	0	0	0	0	0	0	0	0
	0.1	0	0	0	0	20	20	20	30	40	50
	0.5	0	0	0	10	50	50	50	60	60	70
	1.0	0	0	0	0	20	30	30	40	50	70
	2.0	0	0	0	10	20	30	50	70	80	80
15	0	0	0	0	0	0	0	0	0	0	0
	0.1	0	0	0	0	10	30	50	50	50	60
	0.5	0	0	0	0	40	50	60	70	80	80
	1.0	0	0	0	10	60	70	90	90	90	90
	2.0	0	0	0	0	20	50	60	70	70	70

Table 29 Main effect of coconut water concentration on number of protocorm-like bodies of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Coconut water (%)	No. of protocorm-like bodies / plant
0	1.8±3.0
15	2.1±2.4
$LSD_{.05}$	*NS*

NS= not significantly different

Main effect of BAP

BAP had significant effect on number of plbs per plant, but BAP at concentrations of 0.1 to 2.0 mg/l did not give significant difference (Table 30).

Table 30 Main effect of BAP concentration on number of protocorm-like bodies of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

BAP (mg/l)	No. of protocorm-like bodies / plant	
0	0±0	b
0.1	1.8±2.6	a
0.5	3.1±3.6	a
1.0	2.5±2.4	a
2.0	2.2±2.5	a
$LSD_{.05}$	*1.53*	

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05

Interaction effect of coconut water and BAP

After culturing for 10 weeks, adding coconut water in combination with BAP at various concentrations had interaction effect on number of plbs. It was found that plants cultured in the media without BAP both in the absence and presence of coconut water did not produce plbs. In addition, plantlets cultured in the medium without coconut water, but having BAP at 0.5 mg/l gave the best plbs production. But when having coconut water, the plantlets produced highest in number of plbs when adding BAP at higher concentration, i.e. 1.0 mg/l (Table 31).

Table 31 Effects of coconut water and BAP concentrations on number of protocorm-like bodies of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

CW (%)	BAP (mg/l)	No. of protocorm-like bodies / plant	
0	0	0±0	c
	0.1	2.0±3.2	abc
	0.5	4.0±4.8	a
	1.0	1.0±0.9	bc
	2.0	1.8±1.9	bc
15	0	0±0	c
	0.1	1.6±1.8	bc
	0.5	2.2±1.6	ab
	1.0	4.0±2.5	a
	2.0	2.5±3.1	ab
$LSD_{.05}$		*2.17*	

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05

Experiment 4 Effects of IAA, BAP and coconut water concentrations in the dark condition

This experiment was carried out to find the effects of IAA and BAP concentrations, and coconut water on vegetative growth, *in vitro* tuberization and root growth in a dark condition. Their effects on the accumulation of some carbohydrate substances, i.e. total soluble sugar and starch contents, in various plant parts, i.e. shoot and leaf, protocorm and tuber at the end of culturing were also investigated.

4.1 Vegetative growth

After the protocorms were transferred onto the tested medium (without organic substances, except in the control medium; ctrl.) and placed in the dark condition, it was found that plantlets started to emerge young leaf in the first week, but morphological changes of their leaves were different from those cultured in a light condition. In addition, the young leaves extended straight upward and not fully expanded. They had no chlorophyll developing, resulting in producing white leaves. During culturing for 10 weeks, the plantlets cultured in various tested media did not show obvious difference in leaf emerging percentages (Table 32).

Table 32 Effects of IAA, BAP and coconut water concentrations on leaf-emerging percentages of protocorms during culturing for 10 weeks.

Treatment*	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
Ctrl.	90	95	95	95	95	95	95	95	100	100
T1	95	95	100	100	100	100	100	100	100	100
T2	90	90	95	95	95	95	95	95	95	95
T3	90	90	95	95	95	95	95	95	95	95
T4	90	95	95	95	95	95	95	95	95	95
T5	90	95	100	100	100	100	100	100	100	100
T6	85	90	95	100	100	100	100	100	100	100
T7	90	90	100	100	100	100	100	100	100	100
T8	85	90	95	95	95	95	95	95	95	95

* Ctrl.:CMU_1; T1:CMU_1,-organic,-CW; T2:T1+CW; T3,T4,T5 = T1+IAA at 0.01, 0.10 and 1.00 mg/l, respectively; T6,T7,T8 = T1+BAP at 0.01, 0.05 and 0.10 mg/l, respectively.

After culturing for 10 weeks, it showed that the various tested media had no significant effect on shoot height, leaf width and leaf length. However, the tested media gave significant difference in number of leaf per plant, but they had no distinctly relation between types of growth regulator and their concentrations; neither between the medium with or without organic additives (control and T1-medium), nor between without or with coconut water in T1 and T2 media (Table 33).

Table 33 Effects of IAA, BAP and coconut water concentrations on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Treatment	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
Ctrl.: CMU_1	10.9±2.7	1.6±0.5 ab	1.0±0.4	5.0±2.6
T1 : CMU_1,-organic,-CW	12.2±3.4	1.7±0.5 a	0.9±0.3	6.3±2.5
T2 : T1+CW	12.9±3.6	1.5±0.6 abc	1.1±0.6	6.5±2.9
T3 : T1+0.01 IAA	13.6±4.8	1.5±0.6 abc	1.2±0.6	6.5±3.2
T4 : T1+0.10 IAA	12.4±7.1	1.2±0.5 c	1.0±0.5	6.9±6.0
T5 : T1+1.00 IAA	11.2±3.1	1.4±0.5 abc	0.9±0.3	5.5±2.4
T6 : T1+0.01 BAP	12.1±4.8	1.3±0.5 bc	1.1±0.3	6.9±3.3
T7 : T1+0.05 BAP	11.9±3.5	1.7±0.6 a	1.0±0.5	6.1±2.9
T8 : T1+0.10 BAP	10.7±3.9	1.5±0.6 ab	0.9±0.4	5.4±2.6
$LSD_{.05}$	*NS*	*0.34*	*NS*	*NS*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

4.2 Tuberization and growth

It showed that plantlets started to form initial tuber after culturing for 1 week, except in the medium without growth supplements (T1). During culturing for 2 to 4 weeks, it was found that adding BAP at 0.01, 0.05 and 0.10 mg/l (T6, T7 and T8, respectively) supported higher tuberization percentages. However, the plantlets cultured in various tested media showed no obvious difference in tuberization percentage after culturing for 10 weeks (Table 34).

Table 34 Effects of IAA, BAP and coconut water concentrations on tuberization percentages of seedlings during culturing for 10 weeks.

Treatment*	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
Ctrl.	15	30	45	60	80	90	100	100	100	100
T1	0	10	50	80	95	100	100	100	100	100
T2	10	20	30	55	70	90	100	100	100	100
T3	5	30	45	55	70	80	100	100	100	100
T4	5	40	55	75	90	90	95	100	100	100
T5	5	20	35	70	85	90	90	100	100	100
T6	5	55	75	90	95	95	95	95	100	100
T7	5	55	75	90	95	95	95	95	95	95
T8	20	60	85	95	100	100	100	100	100	100

* Ctrl.:CMU_1; T1:CMU_1,-organic,-CW; T2:T1+CW; T3,T4,T5 = T1+IAA at 0.01, 0.10 and 1.00 mg/l, respectively; T6,T7,T8 = T1+BAP at 0.01, 0.05 and 0.10 mg/l, respectively.

After culturing for 10 weeks in various tested media, plantlets did not show significant difference in number of tuber per plant and tuber width, but gave significant difference in their length. In the presence or absence of coconut water, adding IAA at 0.01 – 0.10 mg/l and also BAP at 0.01 – 0.10 mg/l did not show difference in tuber length significantly; but the highest IAA at 1.00 mg/l (T5), the tuber was shortest. Plantlets produced the biggest tuber when cultured in the medium supplemented with BAP at 0.05 and 0.10 mg/l (Table 35 and Figure 7).

For the tuber shape, plantlets cultured in media without organic substances both presence or absence of coconut water (T1 and T2, respectively) gave the highest percentages of oval-shape tuber, but adding both organic substances and coconut water (control), the percentage of oval-shape tuber decreased to 70 %. Moreover, adding IAA especially at 0.10 and 1.00 mg/l, and BAP at all tested concentrations obviously resulted in decreasing percentages of oval-shape tuber formation (Table 35).

Table 35 Effects of IAA, BAP and coconut water concentrations on number of tuber, tuber size and oval-shape tuber percentage of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Treatment	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)	Oval-shape tuber (%)
Ctrl.: CMU_1	1.0±0	2.9±0.8	7.1±2.1 abc	70
T1 : CMU_1,-organic,-CW	1.0±0	2.8±0.5	7.5±1.8 ab	100
T2 : T1+CW	1.1±0.2	2.9±0.6	7.5±2.2 ab	100
T3 : T1+0.01 IAA	1.1±0.2	2.5±0.7	6.8±2.1 abc	80
T4 : T1+0.10 IAA	1.0±0	3.0±0.8	6.5±2.3 bc	65
T5 : T1+1.00 IAA	1.0±0	2.9±1.2	5.6±2.5 c	65
T6 : T1+0.01 BAP	1.1±0.2	2.7±0.8	7.4±2.9 ab	55
T7 : T1+0.05 BAP	1.0±0.2	3.0±1.2	8.2±3.0 a	60
T8 : T1+0.10 BAP	1.0±0	3.2±0.7	8.0±2.6 ab	60
$LSD_{.05}$	*NS*	*NS*	*1.51*	*NA*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different, NA = not statistical analysed

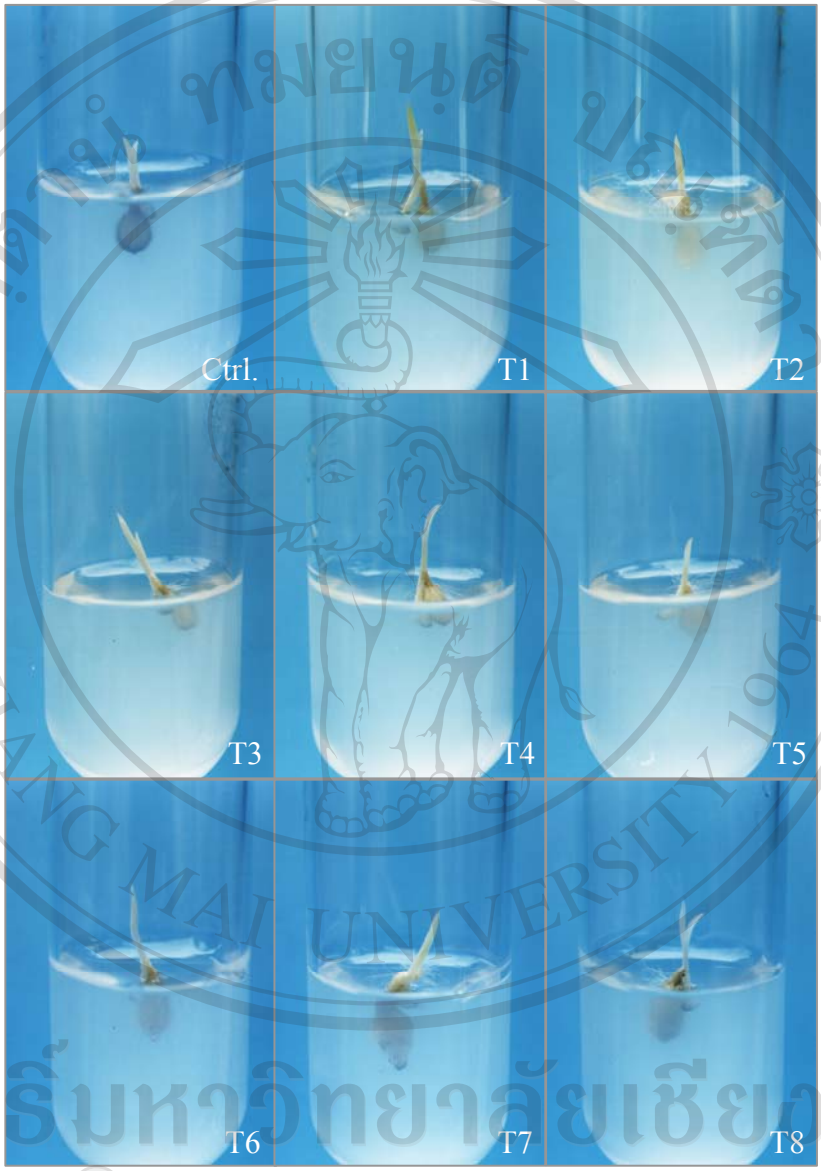


Figure 7 *Pecteilis sagarikii* seedlings cultured in various tested medium in continuous darkness after culturing for 10 weeks.
Ctrl. = CMU_1;
T1 = CMU_1,–organic substances,–CW; T2 = T1+CW 15%
T3, T4 and T5 = T1+IAA at 0.01, 0.10 and 1.00 mg/l, respectively.
T6, T7 and T8 = T1+BAP at 0.01, 0.05 and 0.10 mg/l, respectively.

4.3 Root formation and growth

During culturing for 10 weeks, plantlets cultured in the T8 medium supplemented with BAP at the highest concentration, i.e. 0.10 mg/l showed slow root formation and also gave the lowest rooting percentage. In addition, plantlets cultured in the T1 medium, without organic substances and coconut water, gave rooting percentages less than those cultured in the control medium (Table 36).

Table 36 Effects of IAA, BAP and coconut water concentrations on rooting percentages of seedlings during culturing for 10 weeks.

Treatment*	Time after culturing (weeks)									
	1	2	3	4	5	6	7	8	9	10
Ctrl.	15	25	40	45	45	45	45	45	45	45
T1	0	10	15	25	30	30	30	30	30	30
T2	0	25	35	40	40	40	40	40	40	40
T3	0	15	25	30	35	35	35	35	35	35
T4	10	40	40	45	50	50	50	50	50	50
T5	15	25	40	50	50	50	50	50	50	50
T6	5	25	35	35	35	35	35	35	35	40
T7	15	35	45	50	50	50	50	50	50	50
T8	0	0	15	15	15	15	20	20	25	25

* Ctrl.:CMU_1; T1:CMU_1,-organic,-CW; T2:T1+CW; T3,T4,T5 = T1+IAA at 0.01, 0.10 and 1.00 mg/l, respectively; T6,T7,T8 = T1+BAP at 0.01, 0.05 and 0.10 mg/l, respectively.

After culturing for 10 weeks, plantlets cultured in all treatments showed no significant difference in their number of root and root growth (Table 37).

Table 37 Effects of IAA, BAP and coconut water concentrations on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Treatment	No. of root / plant	Root width (mm)	Root length (mm)
Ctrl.: CMU_1	0.5±0.5	0.3±0.4	1.2±1.9
T1 : CMU_1,-organic,-CW	0.3±0.5	0.2±0.4	0.8±1.4
T2 : T1+CW	0.4±0.5	0.3±0.4	1.0±1.4
T3 : T1+0.01 IAA	0.4±0.6	0.3±0.4	1.0±1.9
T4 : T1+0.10 IAA	0.5±0.5	0.4±0.5	1.6±2.2
T5 : T1+1.00 IAA	0.6±0.6	0.5±0.5	1.6±1.9
T6 : T1+0.01 BAP	0.5±0.6	0.3±0.4	0.8±1.3
T7 : T1+0.05 BAP	0.5±0.5	0.4±0.4	1.2±1.7
T8 : T1+0.10 BAP	0.3±0.4	0.2±0.4	0.4±0.8
$LSD_{.05}$	*NS*	*NS*	*NS*

NS= not significantly different

4.4 Total soluble sugar

Data from total soluble sugar (TSS) analysis in different plant tissues showed that plantlets cultured in T1 medium had more TSS than those cultured in the control treatment being completely supplemented with organic substances and coconut water. While adding only coconut water (T2), an increase of TSS was observed in the tuber, but less in protocorm and shoot parts. For the auxin effect (T3, T4, T5 compared with T1), plantlets had less TSS in protocorm but more in tuber. Less TSS was also observed in shoot except in T5 medium. For the cytokinin effect, BAP at 0.01 mg/l (T6) supported the increases of TSS in shoot and tuber but lesser in protocorm tissues, while BAP at higher concentration at 0.05 and 0.10 mg/l (T7 and T8) decreased TSS in whole plant tissues except in shoot when cultured in T8 medium (Table 38).

Table 38 Effects of IAA, BAP and coconut water concentrations on total soluble sugar in various tissues of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Treatment	Total soluble sugar (µg/g FW.)		
	Protocorm	Shoot	Tuber
Ctrl.: CMU_1	28,955	25,954	24,355
T1 : CMU_1,-organic,-CW	110,377	29,208	38,267
T2 : T1+CW	63,448	23,168	42,582
T3 : T1+0.01 IAA	85,981	24,647	42,572
T4 : T1+0.10 IAA	62,074	26,652	42,910
T5 : T1+1.00 IAA	98,896	37,558	39,331
T6 : T1+0.01 BAP	104,856	34,993	39,757
T7 : T1+0.05 BAP	34,822	26,637	25,742
T8 : T1+0.10 BAP	26,676	30,190	21,438

Remark: The TSS content in the protocorm from the initial culture was 21,900±9,780 µg/g FW.

4.5 Starch accumulation

Plantlets cultured in the medium lacking of organic additives and coconut water (T1) gave more starch contents in their protocorms and shoots than those culturing in the control, but adding only coconut water (T2) gave increased starch content in all plant tissues. For the auxin effect (T3, T4, T5 compared with T1), IAA gave high starch contents in both protocorms and shoots, while, in the tuber, IAA at 0.01 to 0.10 mg/l (T3 and T4) supported a decrease in starch contents, but when the highest level at 1.0 mg/l (T5) was added, it gave a positive effect to increase in starch contents. For the cytokinin effect (T6, T7, T8 compared with T1), BAP had obviously effected to decrease in starch contents in protocorms and tubers, but when applied at the lowest concentration at 0.01 mg/l (T6), it gave more positive effect in increasing starch content in shoot. The very high starch accumulations were found when coconut water (T2) or IAA at 1.0 mg/l (T5) was added (Table 39).

Table 39 Effects of IAA, BAP and coconut water concentrations on starch contents in various tissues of *Pecteilis sagarikii* seedlings after culturing for 10 weeks.

Treatment	Starch content (µg/g FW.)		
	Protocorm	Shoot	Tuber
Ctrl.: CMU_1	8,946	2,584	26,540
T1 : CMU_1,-organic,-CW	61,074	1,659	38,013
T2 : T1+CW	129,440	2,879	73,590
T3 : T1+0.01 IAA	68,268	4,188	29,384
T4 : T1+0.10 IAA	75,302	2,823	35,411
T5 : T1+1.00 IAA	202,965	6,131	41,279
T6 : T1+0.01 BAP	33,319	6,802	17,390
T7 : T1+0.05 BAP	13,573	1,652	6,019
T8 : T1+0.10 BAP	20,962	2,363	5,175

Remark: Starch content in the protocorm from the initial culture was 28,995±5,475 µg/g FW.

Staining longitudinal cutting of seedlings with iodine solution showed starch granules distribution in plantlet tissues. Plantlets accumulated high density of starch granules in their protocorms and tubers when cultured in the medium having coconut water (T2), and/or adding IAA at 1.00 mg/l (T5), but showed the lowest density in the tuber tissues from the medium having BAP at high concentrations at 0.05 and 0.10 mg/l (T7 and T8), respectively (Figure 8).

Figure 8 Longitudinal cut of *Pecteilis sagarikii* seedlings after staining with iodine solution, showing starch accumulation in different parts.
Ctrl. = CMU_1
T1 = CMU_1,–organic substances,–CW; T2 = T1+CW 15%
T3, T4 and T5 = T1+IAA at 0.01, 0.10 and 1.00 mg/l, respectively.
T6, T7 and T8 = T1+BAP at 0.01, 0.05 and 0.10 mg/l, respectively.

Experiment 5 Effects of illumination and sucrose concentration

This experiment was carried out to find the effects of illumination and sucrose concentrations, and their interaction on vegetative growth, tuberization and root growth, in the absence of both growth regulator and indirect additive of other carbon sources, e.g. coconut water. Moreover, the harvested cultures were also used to study on their accumulation of some carbohydrate substances, i.e. total soluble sugar and starch contents.

5.1 Vegetative growth

After the uniform protocorms were transferred onto the CMU_1 medium having different concentrations of sucrose and then placed in different illuminating conditions, it was found that plantlets cultured in a light condition started to emerge their leaves after culturing for 2 weeks, while culturing in a dark condition the leaves occurred 1 week later. High sucrose concentration, i.e. at 5 % in the dark condition, or at 3 to 5 % in the light condition, helped to slightly decrease leaf emerging percentage (Table 40).

In addition, the colors of the plantlets culturing in different illumination conditions were different. Those plantlets culturing in the dark were still white, whereas in the light condition, they developed chlorophyll resulting in green leaves (Figure 9).

Table 40 Effects of illumination and sucrose concentration on leaf-emerging percentages of *Pecteilis sagarikii* seedlings during culturing for 12 weeks.

Illumination	Su. (%)	Time after culturing (weeks)											
		1	2	3	4	5	6	7	8	9	10	11	12
Continuous Dark	0	0	0	30	75	80	80	80	80	80	80	80	80
	1	0	0	50	65	80	80	80	80	80	80	80	80
	2	0	0	75	80	85	85	85	85	85	85	85	85
	3	0	0	55	65	70	70	70	70	70	70	70	70
	5	0	0	20	35	35	40	40	40	40	45	45	45
Light 16 h/d	0	0	10	50	55	60	60	60	60	60	60	60	60
	1	0	5	50	55	60	60	65	65	65	65	65	65
	2	0	15	40	40	60	60	60	60	60	60	60	60
	3	0	15	25	30	35	35	35	40	40	40	40	40
	5	0	10	30	40	40	40	45	45	45	45	45	45

Main effect of illumination

It was found that illumination had significant effects on shoot height and leaf length but had no significant effects on the number of leaf per plant, and leaf width. In addition, the plantlets cultured in the dark condition gave more shoot height and also leaf length than those cultured in the light condition (Table 41).

Table 41 Main effect of illumination on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
Continuous Dark	11.6±4.6 a	0.8±0.5	0.4±0.3	3.5±4.0 a
Light 16h/d	8.9±2.0 b	0.7±0.7	0.6±0.7	1.4±1.7 b
$LSD_{.05}$	*0.97*	*NS*	*NS*	*0.85*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Main effect of sucrose

It was found that concentration of sucrose had no significant effect on shoot height and leaf size but significantly affected the number of leaf per plant. Adding sucrose at 0, 1 and 2 % gave better in number of leaf per plant than those in higher concentrations. Moreover, adding sucrose at 1 and 2 % gave better trend in shoot height and leaf size than adding sucrose at higher levels (Table 42).

Table 42 Main effect of sucrose concentration on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Sucrose (%)	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
0	9.8±3.0	0.8±0.5 abc	0.6±0.5	2.8±2.6
1	10.4±3.9	0.9±0.7 a	0.6±0.7	2.9±3.2
2	11.5±4.7	0.9±0.6 ab	0.5±0.5	3.1±4.0
3	9.8±2.6	0.6±0.6 bc	0.4±0.4	1.9±2.3
5	9.8±4.2	0.5±0.6 c	0.4±0.6	1.6±3.7
$LSD_{.05}$	*NS*	*0.27*	*NS*	*NS*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Interaction effect of illumination and sucrose

After culturing for 12 weeks, it showed that illumination and sucrose had interaction effect on shoot height, number of leaf per plant and leaf length but had no significant effect on leaf width. In addition, those plantlets cultured in the dark condition gave shoot height more than from the light condition, especially when cultured in the medium having sucrose concentrations at 1 or 2 %. For number of leaf per plant, it was found that the highest numbers of leaf were produced in the medium adding sucrose at 0 % to 3 % in the dark condition, but in the light condition best result was obtained from in the medium having sucrose at lesser concentrations, i.e. 1 and 2 % or no sucrose. Moreover, plantlets cultured in the dark condition have more leaf length than that from the light condition, but sucrose at high concentrations, i.e. at 3 and 5 % in the dark condition and/or at 2 to 5 % in light condition, reduced their leaf length. The plantlets grew well when cultured in the dark condition on the medium

adding sucrose at 2 %, but in the light condition, they grew well when sucrose was added at only 1 % (Table 43).

Table 43 Effects of illumination and sucrose concentration on shoot height, number of leaf and leaf size of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	Su. (%)	Shoot height (mm)	No. of Leaf / plant	Leaf width (mm)	Leaf length (mm)
Continuous Dark	0	10.9±3.5 bc	0.8±0.4 ab	0.5±0.3	3.7±3.1 ab
	1	12.3±4.5 ab	0.8±0.4 ab	0.4±0.3	3.7±3.7 ab
	2	13.8±5.2 a	1.0±0.6 a	0.6±0.3	5.0±4.8 a
	3	10.8±2.9 bc	0.8±0.6 ab	0.4±0.3	2.9±2.7 bc
	5	10.3±5.6 bcd	0.5±0.5 b	0.3±0.4	2.1±5.1 bcd
Light 16 h/d	0	8.8±2.0 cd	0.7±0.7 ab	0.7±0.7	1.9±1.7 bcd
	1	8.5±1.8 d	1.1±0.9 a	0.8±0.9	2.2±2.5 bcd
	2	9.1±2.4 cd	0.7±0.7 ab	0.5±0.6	1.1±1.1 cd
	3	8.7±1.8 cd	0.5±0.6 b	0.3±0.5	0.9±1.3 d
	5	9.3±2.0 cd	0.6±0.7 b	0.5±0.7	1.1±1.5 cd
$LSD_{.05}$		*2.16*	*0.38*	*NS*	*1.90*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

5.2 Tuberization and growth

The cultured plantlets generally started to show tuberization after culturing for 2 weeks both in the dark and light conditions, except in some treatments that occurred in the third week. During culturing for 12 weeks, those plantlets cultured in the dark condition provided more tuberization percentage in all the sucrose-containing media. While cultured in the light condition, plantlets could form tuber well although they were cultured in the absence of sucrose.

After culturing for 12 weeks, the plantlets cultured in the sucrose-adding medium both in the dark and light conditions gave higher tuberization percentages than those cultured in the medium without sucrose. In addition, plantlets cultured in the dark condition gave better tuberization than that from the light condition, when the media having sucrose at 1 % and 3 %. But when cultured in the medium without sucrose the plantlets produced more tuberization percentage in the light condition than those cultured in the continuous darkness (Table 44).

Table 44 Effects of illumination and sucrose concentrations on tuberization percentages of *Pecteilis sagarikii* seedlings during culturing for 12 weeks.

Illumination	Su. (%)	Time after culturing (weeks)											
		1	2	3	4	5	6	7	8	9	10	11	12
Continuous Dark	0	0	5	15	30	45	45	50	70	70	75	75	75
	1	0	0	40	65	75	80	85	95	95	100	100	100
	2	0	0	20	55	70	80	90	95	100	100	100	100
	3	0	10	30	45	50	65	90	95	100	100	100	100
	5	0	20	30	50	65	70	80	90	90	90	90	95
Light 16 h/d	0	0	20	40	65	75	80	85	85	85	85	85	85
	1	0	0	10	35	60	70	85	85	90	90	90	90
	2	0	15	70	75	75	85	90	95	100	100	100	100
	3	0	15	30	50	60	70	75	75	90	90	90	90
	5	0	5	15	35	45	65	80	85	90	90	95	95

Main effect of illumination

It showed that illumination had no significant effect on the number of tuber per plant but showed significant effects on tuber width and tuber length. In addition, the tubers produced in the dark condition were wider but shorter than those produced in the light condition (Table 45).

Table 45 Main effect of illumination on number of tuber and tuber size of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)
Continuous Dark	1.0±0.3	2.5±1.2 a	7.3±3.2 b
Light 16h/d	0.9±0.3	1.9±1.1 b	8.4±5.2 a
$LSD_{.05}$	*NS*	*0.26*	*1.07*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Main effect of sucrose

Concentration of sucrose had significant effect on the number of tuber per plant and tuber size. The plantlets cultured in the medium having sucrose gave more number of tubers than those cultured in the medium without sucrose. However, there were no significant differences among the sucrose concentration at 1 to 5 %. For tuber size, adding sucrose at 1 to 5 % gave bigger tuber size than that obtained from the medium in the absence of sucrose, while a low sucrose concentration at 1 % supported a decrease in tuber width. However, adding sucrose at the highest concentration gave negative effect on tuber size, i.e. sucrose at 5% affected in decreasing tuber width, and sucrose at 3 and 5 % suppressed growth in terms of the tuber length. The best tuber growth was obtained from the medium having sucrose at 2 % (Table 46).

Table 46 Main effect of sucrose concentration on number of tuber and tuber size of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Sucrose (%)	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)
0	0.8±0.4 b	1.2±0.9 d	5.2±4.7 d
1	1.0±0.2 a	2.3±1.0 bc	8.8±4.6 ab
2	1.0±0 a	2.7±0.9 a	10.0±2.9 a
3	1.0±0.2 a	2.6±1.1 ab	8.1±4.1 bc
5	1.0±0.3 a	2.1±1.2 c	7.1±3.7 c
$LSD_{.05}$	*0.11*	*0.41*	*1.70*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05

Interaction effect of illumination and sucrose

Illumination and sucrose concentrations had interaction effect on the number of tuber and tuber size. The plantlets cultured in the dark condition gave the best result in the number of tuber when cultured in the media having sucrose at 1 to 5 %, but when light was given, they did not show a significant effect of sucrose concentrations although they were cultured in the medium without sucrose. For tuber growth, the cultured tubers started to decrease in growth when cultured in the medium having 1 % sucrose or without it in the dark condition. But culturing in light, they showed a significant decrease in tuber growth when cultured in the medium without or with sucrose at 1 % or at high concentrations, i.e. 3 and 5 %. Interestingly, it was found that tuber grew well and produced the biggest size when cultured in the medium adding 3 % sucrose in darkness, but when cultured in the light condition, an optimal sucrose concentration was 2 % (Table 47 and Figure 9).

For the tuber shape, it showed that those plantlets cultured in the dark condition produced more oval-shape tubers than those culturing in the light condition, except in the medium without sucrose (Table 47).

Table 47 Effects of illumination and sucrose concentration on number of tuber and tuber size of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	Su. (%)	No. of tuber / plant	Tuber width (mm)	Tuber length (mm)	Oval-shape tuber (%)
Continuous Dark	0	0.8±0.4 b	0.8±0.7 e	2.7±2.0 e	45
	1	1.0±0 a	2.6±0.8 b	8.1±2.1 bcd	95
	2	1.0±0 a	3.0±0.9 ab	8.8±1.8 abcd	95
	3	1.0±0 a	3.2±0.6 a	9.2±2.0 abc	95
	5	1.0±0.3 a	2.7±1.0 ab	7.6±2.7 bcd	90
Light 16 h/d	0	0.9±0.4 ab	1.6±1.0 d	7.7±5.3 bcd	50
	1	0.9±0.3 ab	2.0±1.1 cd	9.6±6.1 ab	65
	2	1.0±0 a	2.5±0.9 bc	11.1±3.4 a	60
	3	0.9±0.3 ab	1.9±1.1 d	7.1±5.3 cd	55
	5	1.0±0.2 a	1.6±1.1 d	6.5±4.4 d	45
$LSD_{.05}$		*0.16*	*0.58*	*2.40*	*NA*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NA = not statistical analysed

5.3 Root formation and growth

The cultured plantlets started to root after culturing for 2 weeks, except in some treatments which occurred in the third week. After culturing for 12 weeks, those cultured in the dark condition with the medium having different sucrose concentrations including in the absence of sucrose gave better rooting percentages than those cultured in the light condition. In addition, in the light condition the plantlets gave a decrease in the rooting percentage when cultured in the medium having sucrose at the highest level at 5 %, and then gave the least rooting percentage in the medium devoid of sucrose (Table 48).

Table 48 Effects of illumination and sucrose concentrations on rooting percentages of *Pecteilis sagarikii* seedlings during culturing for 12 weeks.

Illumination	Su. (%)	Time after culturing (weeks)											
		1	2	3	4	5	6	7	8	9	10	11	12
Continuous Dark	0	0	5	25	35	35	45	50	50	50	50	50	50
	1	0	20	40	45	50	50	55	60	60	60	60	60
	2	0	15	35	45	50	50	50	50	50	50	50	50
	3	0	10	25	45	50	50	50	50	50	50	50	50
	5	0	0	20	35	40	45	50	55	55	55	55	55
Light 16 h/d	0	0	5	10	10	15	15	15	15	20	20	20	20
	1	0	5	35	40	45	45	45	45	45	45	45	45
	2	0	15	35	40	40	40	40	40	40	40	40	40
	3	0	0	20	20	20	35	40	40	40	40	40	40
	5	0	5	30	30	30	30	35	35	35	35	35	35

Main effect of illumination

It was found that illumination had a significant effect on the number of roots per plant and root width but had no effect on root length. The plantlets cultured in the dark condition gave more roots per plant and root width than those cultured in the light condition (Table 49).

Table 49 Main effect of illumination on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	No. of root / plant	Root width (mm)	Root length (mm)
Continuous Dark	0.6±0.6 a	0.4±0.4 a	1.3±1.6
Light 16h/d	0.4±0.5 b	0.3±0.4 b	1.1±2.1
$LSD_{.05}$	*0.16*	*0.10*	*NS*

Means within the same column followed by different characters showed significantly different by LSD test at P≤ .05, NS= not significantly different

Main effect of sucrose

Sucrose concentrations had no significant effect on the number of root, root width, nor root length (Table 50).

Table 50 Main effect of sucrose concentrations on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Sucrose (%)	No. of root / plant	Root width (mm)	Root length (mm)
0	0.4±0.5	0.2±0.3	0.6±1.1
1	0.5±0.5	0.4±0.4	1.5±2.6
2	0.5±0.6	0.3±0.4	1.4±1.8
3	0.5±0.6	0.4±0.4	1.2±1.6
5	0.5±0.6	0.3±0.4	1.3±1.9
$LSD_{.05}$	*NS*	*NS*	*NS*

NS= not significantly different

Interaction effect of illumination and sucrose

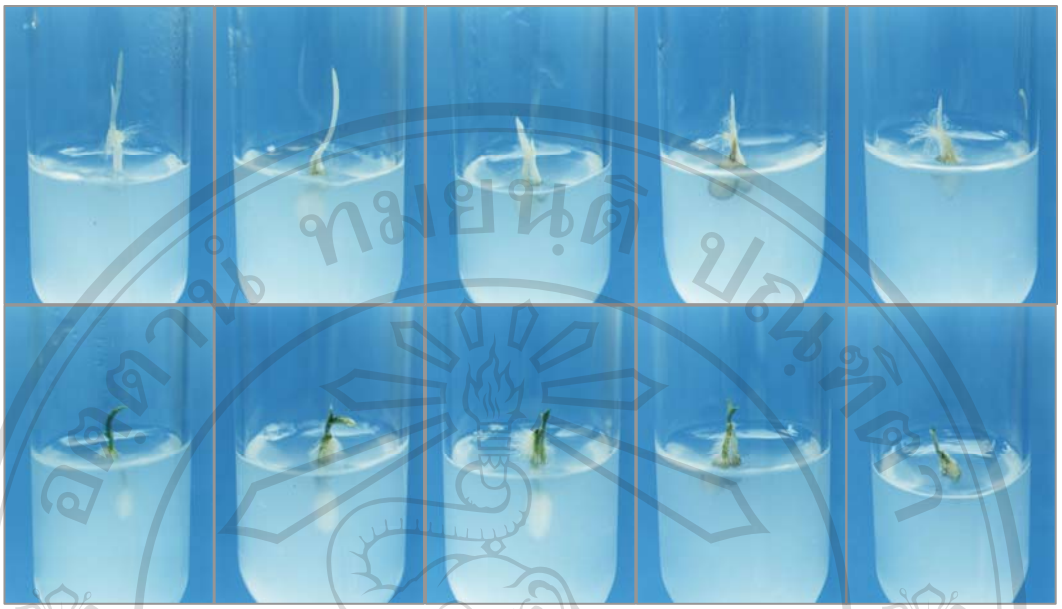
Illumination and sucrose had no significant interaction effect on the number of root, root width and root length after culturing for 12 weeks (Table 51).

Table 51 Effects of illumination and sucrose concentrations on number of root, root width and root length of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	Su. (%)	No. of root / plant	Root width (mm)	Root length (mm)
Continuous Dark	0	0.5±0.5	0.3±0.4	0.6±0.7
	1	0.6±0.5	0.4±0.3	1.0±1.0
	2	0.6±0.7	0.4±0.4	1.5±1.7
	3	0.6±0.6	0.4±0.4	1.5±1.8
	5	0.6±0.6	0.4±0.4	1.9±2.3
Light 16 h/d	0	0.2±0.4	0.1±0.3	0.6±1.5
	1	0.5±0.5	0.4±0.5	2.0±3.5
	2	0.4±0.5	0.3±0.4	1.3±1.9
	3	0.5±0.7	0.3±0.4	1.0±1.4
	5	0.4±0.5	0.2±0.3	0.7±1.2
$LSD_{.05}$		*NS*	*NS*	*NS*

NS= not significantly different

A



B

Figure 9 *Pecteilis sagarikii* seedlings cultured in two light conditions.
A) Continuous darkness B) Light for 16 hours/day
The cultures were in CMU_1 medium adding sucrose at different concentrations at 0, 1, 2, 3 and 5 % (from left to right) for 12 weeks.

5.4 Total soluble sugar

After harvesting, the plantlets showed the increases of TSS in their protocorms, shoots and tubers from the cultures grown in the media with increasing sucrose concentrations both in the dark and light conditions, except in their tubers culturing in the dark condition with the highest sucrose concentration. When the effects of the same sucrose concentrations were compared, the plantlets had higher TSS contents in shoots and tubers when cultured in the light condition, except in their tubers in the medium without sucrose; while the plantlets showed the increases of TSS in their protocorms when cultured in light condition in the medium adding sucrose at 0, 1 and 5 % but decreased when cultured in the medium adding sucrose at 2 % and 3 % (Table 52).

5.5 Starch accumulation

The plantlets from the light condition accumulated more starch in their shoots than those cultured in the dark condition, when compared with the same level of sucrose. This effect was similarly found in the protocorm tissues, except in the medium having 2 % sucrose that showed an increase in the starch content when cultured in the dark condition. In tuber, it showed that the plantlets increased in starch accumulation in the light condition when cultured in the medium without

sucrose and the medium with the highest sucrose concentration at 5 %. But when having sucrose at 1 to 3 %, they gave better starch accumulation in the dark condition.

The starch accumulation in the protocorms, shoots and tubers cultured in the same light condition was compared. It showed that the accumulation obviously increased with increasing sucrose levels; especially at the highest concentration, i.e. 5 % except in a few treatments (Table 53).

Table 52 Effects of illumination and sucrose concentrations on total soluble sugar in various tissues of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	Su. (%)	Total soluble sugar (µg/g FW.)		
		Protocorm	Shoot	Tuber
Continuous Dark	0	3,607	4,721	9,823
	1	7,190	8,226	9,865
	2	20,393	9,284	13,627
	3	37,705	21,060	23,086
	5	39,354	28,138	22,397
Light 16 h/d	0	5,732	14,683	6,584
	1	12,013	18,017	14,316
	2	15,909	19,129	18,920
	3	32,111	39,558	26,247
	5	45,590	60,000	39,920

Remark: The TSS content in the protocorm from the initial culture was 21,900±9,780 µg/g FW.

Table 53 Effects of illumination and sucrose concentrations on starch contents in various tissues of *Pecteilis sagarikii* seedlings after culturing for 12 weeks.

Illumination	Su. (%)	Starch content (µg/g FW.)		
		Protocorm	Shoot	Tuber
Continuous Dark	0	2,090	3,049	6,737
	1	1,298	4,531	36,391
	2	63,343	4,124	52,532
	3	20,372	5,526	61,346
	5	84,938	6,937	100,768
Light 16 h/d	0	4,147	6,661	8,781
	1	31,897	8,630	12,334
	2	56,351	18,750	36,005
	3	90,320	26,127	58,240
	5	151,937	18,124	106,754

Remark: Starch content in the protocorm from the initial culture was 28,995±5,475 µg/g FW.

An observation on starch granule distribution in different plant organs using iodine staining showed that the plantlets increased the density of starch granules, especially in their protocorms and tubers when cultured in the medium with increasing sucrose concentrations both in the dark and light conditions (Figure 10).

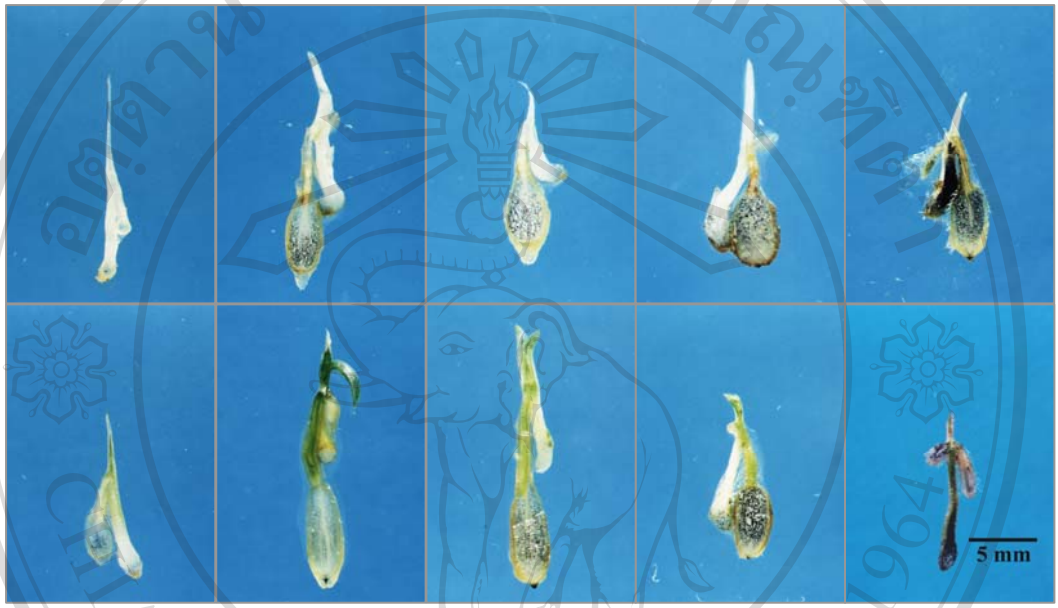


Figure 10 The longitudinal free-hand sections of *Pecteilis sagarikii* seedlings staining with iodine solution showing starch accumulation in their different plant parts.
The cultures were in two light conditions. A) continuous darkness B) light for 16 hours/day in CMU_1 medium adding sucrose at different concentrations at 0, 1, 2, 3 and 5 % (from left to right) for 12 weeks.

Conclusion results in Part I

Experiment 1, plantlets produced the biggest leaf size when cultured in CMU_1 medium supplemented with sucrose at 1 % or without sucrose, but it gave the biggest tuber size in 1% sucrose level.

Experiment 2, culturing the protocorms in a medium with or without coconut water, did not show significant difference in shoot growth; but coconut water at 5 – 20 % gave more tuber width than that obtained from the absence coconut water.

Experiment 3, coconut water at 15 % gave better shoot and tuber growth. It had interaction effects with BAP on tuber formation. Increasing BAP affected a decrease in number of tuber, tuber size and oval-shape tuber forming percentage; but when combined with coconut water, better result on tuber formation was obtained. BAP induced plbs formation, an optimal level of BAP was 0.5 mg/l; but in the presence of coconut water, the optimal BAP level was changed to 1.0 mg/l.

Experiment 4, in the dark condition, adding coconut water, IAA at 0.01 – 1.00 mg/l or BAP at 0.01 – 0.10 mg/l did not effect shoot and tuber growth, except in IAA at 1.00 mg/l which affected a decrease in tuber length. Both IAA and BAP reduced percentage of oval-shape tuber formation. Coconut water supported the highest starch accumulation in tuber but IAA and BAP, in general, reduced starch accumulation.

Experiment 5, the dark condition promoted an increase in tuber width, while the light condition promoted tuber length. In the absence of coconut water in the used basal medium, illumination and sucrose concentration had interaction effects on tuber width and length, and also the percentage of oval-shape tuber formation. In the dark condition the biggest tuber was produced when cultured in the medium with 3 % sucrose, while in the light condition, the optimal sucrose concentration was 2 %. Oval-shape tubers were formed at the highest percentage when cultured in the dark condition. Increasing sucrose concentration increased starch accumulation in the cultured protocorms, shoots and tubers, while plant exposing to light provided an increase in starch contents in protocorms and shoots; while tuber, in general, accumulated starch well in the dark condition.

Part II Histological study on tuberization.

1. The first tuberization of a seedling

The study in this section was on the histological changes during *in vitro* tuberization of the cultured seedlings that first occurred after seed germination. When the seven-week-old seeds were sown on the CMU_1 medium and cultured under a dark condition, it showed that their embryos started to grow (enlarged in size) and germinated (burst out from the seed coat) in the 16^{th} week after seed sowing. The sowing seeds did not germinate at the same time, after the first germination the other seeds continued to increase in germination and development. Thus, different stages of the germinated embryo developing into various stages of protocorms could be found at the same time (Figures 11 and 12).

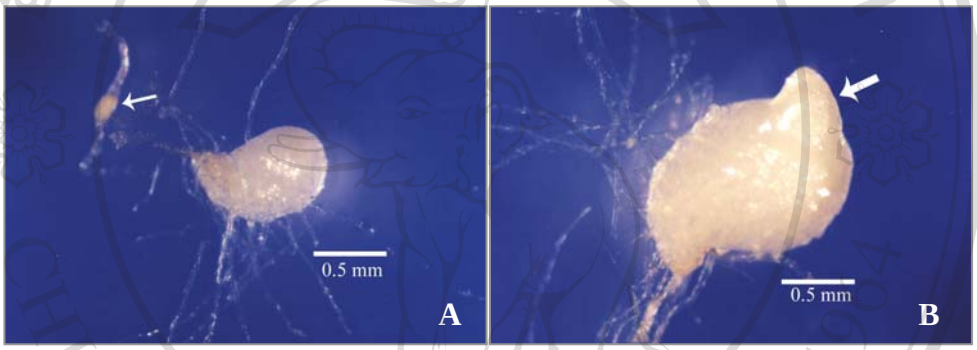


Figure 11 Various stages of *Pecteilis sagarikii* development observed under a stereomicroscope, 20 weeks after seed sowing.
A) A swollen embryo (arrow) and a germinating embryo
B) A protocorm producing a young shoot (arrow)

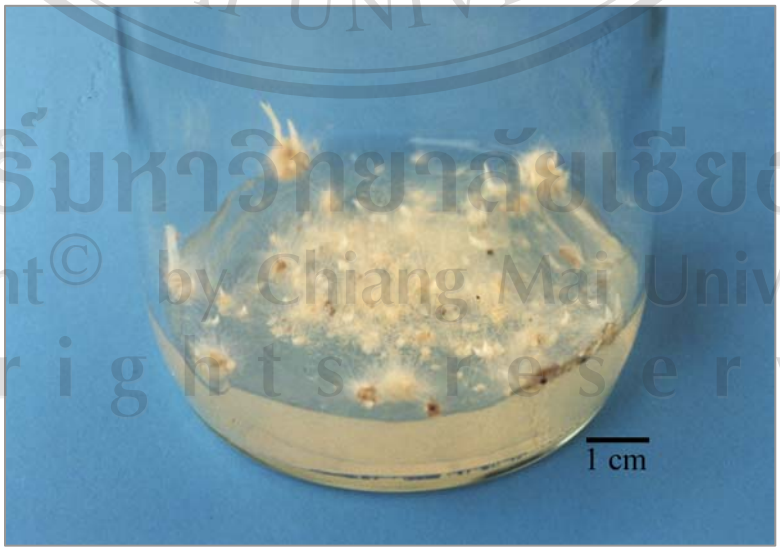


Figure 12 *Pecteilis sagarikii* showed various stages of seedlings in a culture vessel, 20 weeks after seed sowing.

For this study, the samples were chosen from one pod that provided well-uniform germination during the 22nd to the 32nd weeks after seed sowing. During this culture period the initial tuberization stage until the later stages, and some plantlets could be detected. Various stages of the protocorms and plantlets were collected, fixed and then prepared for histological study by using paraffin embedding technique or observing very thin fresh slides by using a freezing microtome.

Morphological changes during the *in vitro* tuberization of the shoot-developing protocorms were observed under a light microscope. It was found that they could be divided into 4 stages as follows:

Stage 1 The formation of a tuber primordium
Stage 2 The extension of the tuber primordium
Stage 3 The formation of a tuber-shoot bud
Stage 4 The enlargement of the tuber

Stage 1 The formation of a tuber primordium

After the sown seeds germinated and developed into the protocorms, which had already developed their shoot meristem and produced some leaf primordia; the meristematic cells especially at the base of the shoot meristem began to divide and started to grow at the front of the protocorm. At this stage, both the shoot meristem of the protocorm and the newly-formed lower meristem started to develop a tuber primordium (Figure 13).

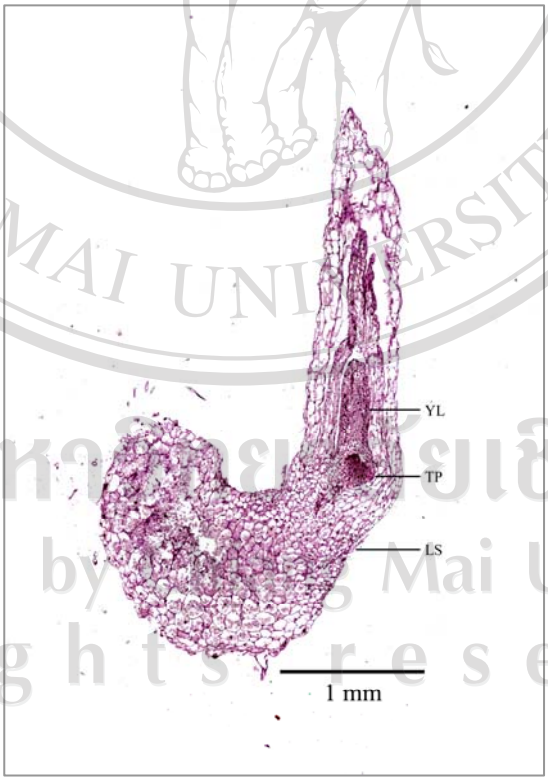


Figure 13 A longitudinal section of *Pecteilis sagarikii* seedling showing a tuber primordium in the early stage of tuberization.
YL = Young leaf; TP = Tuber primordium; LS = Leaf sheath

Stage 2 The extension of the tuber primordium

After the tuber primordium had been completely developed, the meristematic cells of the tuber primordium continued to divide and grew into the opposite direction of its protocorm. The growing cells at the base of developing leaf primordia were simultaneously pushing the tuber primordium extended straight down following the geotropic force and then formed a narrow hollow above the shoot meristem of the tuber primordium. At this stage, the tuber primordium began to emerge in front of the seedling passing through the lower leaf sheath at the joining position between the young shoot and the protocorm body. The tuber primordium continued to grow straight down and produced a tuber stalk (Figure 14).

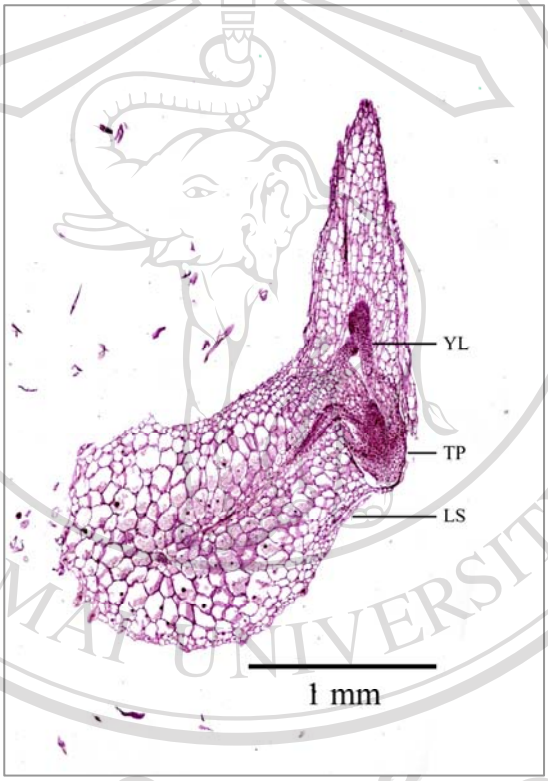


Figure 14 A longitudinal section of *Pecteilis sagarikii* seedling showing a tuber primordium began to grow downwards.
YL = Young leaf; TP = Tuber primordium; LS = Leaf sheath

Stage 3 The formation of a tuber-shoot bud

Subsequently, when the tuber primordium produced the tuber stalk and reached an optimal depth; the meristematic tissue distinctly separated into two growing meristems, i.e. 1) the upper meristem which grew negatively against the geotropic force and produced new leaf primordia to cover its apical meristem, which later completely formed a new tuber-shoot bud for the next growing season; and 2) the lower meristem which continuously divided downwards to form a tuber tip. The

new tuber-shoot bud was formed in the distal cavity at the base of the tuber stalk, and usually pointed to the front of the plant. It had vascular tissue growing downwards from the protocorm body passing through the tuber stalk to the new tuber-shoot bud, and another new vascular tissue existing downward to the lower meristematic cells of the tuber tip (Figure 15).

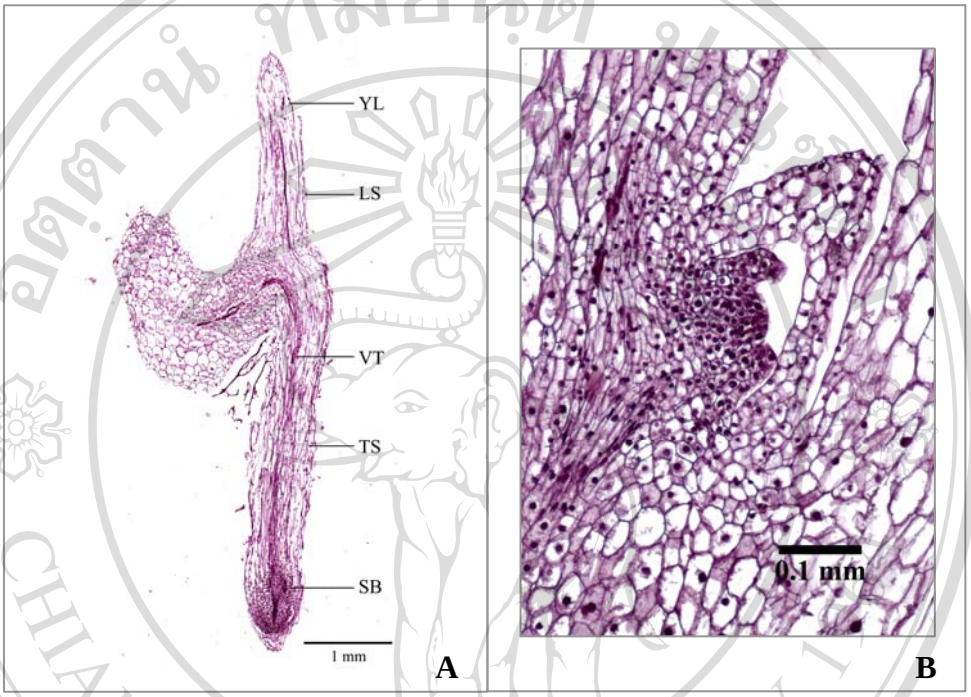


Figure 15 A longitudinal section of *Pecteilis sagarikii* seedling showing a tuber stalk extension and a shoot bud formation.
YL = Young leaf; LS = Leaf sheath; VT = Vascular tissue;
TS = Tuber stalk; SB = Shoot bud
A) A tuber stalk extended straight down into an optimum depth
B) A magnified new shoot bud showing a meristem covered with new leaf primordia (photograph from another sample at the same stage)

Stage 4 The enlargement of a tuber

The final stage of tuber formation, the lower meristematic cells of the tuber tip continued to divide and grew straight down to increase the tuber length and also produced the vascular tissue that developed to form the stele, probably having 1 to 3 steles. During this stage, the cells surrounding the steles, continued to divide and enlarged in size resulting in the oval-shape tuber expansion. Moreover, the tuber started to accumulate some starch granules in their cells, especially in the position near the steles and the meristematic cells both in the new tuber-shoot bud and the tuber tip. These procedures occurred continuously until a complete tuber was obtained (Figures 16 and 17).

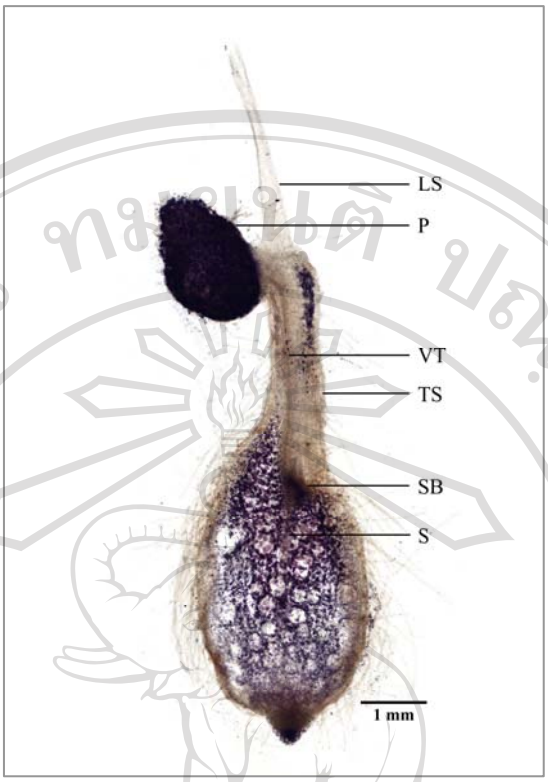


Figure 16 A longitudinal section of *Pecteilis sagarikii* seedling by using a freezing-microtome and staining with iodine solution showing a tuber expansion and starch accumulation in different parts.
LS = Leaf sheath; P = Protocorm; VT = Vascular tissue;
TS = Tuber stalk; SB = Shoot bud; S = Stele

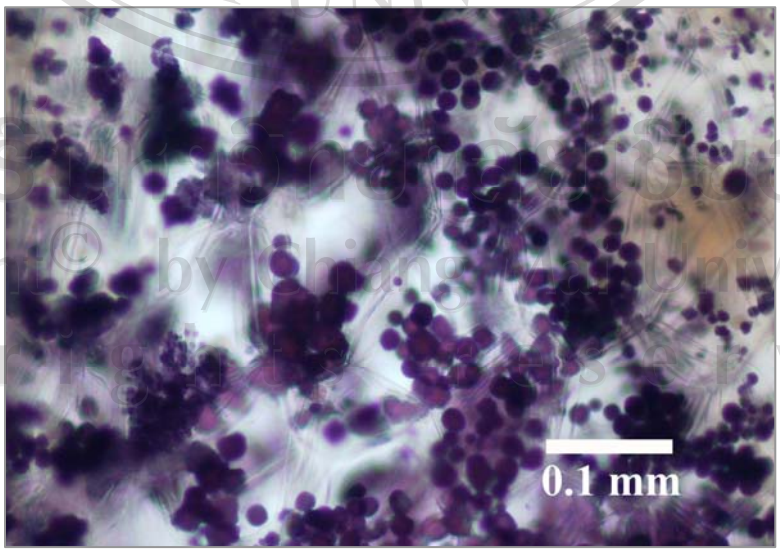


Figure 17 The magnified tuber cells showing various sizes of starch granules.

2. The tuberization of a mature plant

The study in this section was on histological changes during tuberization of a mature plant growing in a greenhouse. Different stages of the plant growth were carefully observed to find the relationship between the external changing of shoot, changing of the shoot tip and the origination of a tuber primordium. It was found that after an early sprouting tuber (Figure 18) was grown, the leafy shoot producing from the shoot tip emerged above the growing medium and continued to expand its leaves until having about 2 – 3 big expanded leaves, which later was defined as the vegetative stage (Figure 19). At the end of the vegetative stage, a complete adventitious bud, at the lowest part of the plant monopodial stem grew until having its size about 1 x 1 mm in width and height, which eventually formed a tuber primordium (Figure 19 B). After that, the shoot of the growing plant produced a very small leaf bract which could be observed at the center part of the plant. When cut to open the shoot, it showed that the apical meristem still produced leaf primordia. At this stage, the tuber primordium continued to grow and increased in its size. This stage of the observed plant can be defined as the pre-flowering stage (Figure 20). Subsequently, in the reproductive stage, the growing plant appeared 2 - 3 small leaf bracts inside its central part, when carefully cut to open the shoot, it showed that the shoot meristem of the plant had already started to differentiate into floret buds (Figure 21c), which eventually continued to develop to complete the floral bud development process. Observing the changing of the tuber primordium, it simultaneously started to grow and extended straight down. After the tuber primordium reached an optimal depth, it produced a new tuber-shoot bud at the upper part of the new tuber, while the meristem at the tuber primordial tip continued to grow straight down to increase in the tuber size (Figure 22). Subsequently, during the development of the mother plant, a young floral spike emerged (Figure 23) and continued to grow until a complete floral spike had been developed and its florets opened, followed by seed-pod setting, until the mother plant wilted. At this period, the tuber grew and increased in its size until a complete tuber was obtained (Figure 24).

Figure 18 A tuber of *Pecteilis sagarikii* in the early sprouting stage.

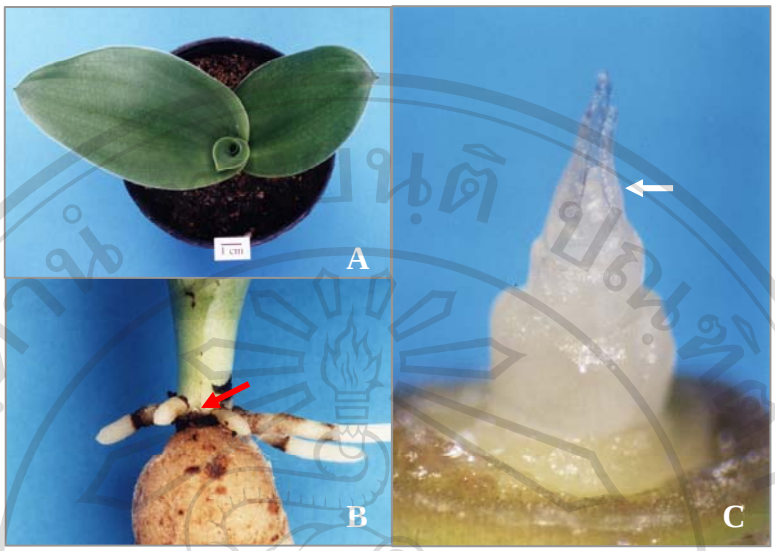


Figure 19 The vegetative stage of *Pecteilis sagarikii*.
A. A whole plant
B. An adventitious bud at the lowest part of the plant forming a tuber primordium (arrow)
C. A shoot tip with young leaf bracts (arrow)

Figure 20 The pre-flowering stage of *Pecteilis sagarikii*.
A. A very small leaf bract occurred inside the central part of a mature plant (arrow)
B. A tuber primordium started to grow and increased in its size (arrow)

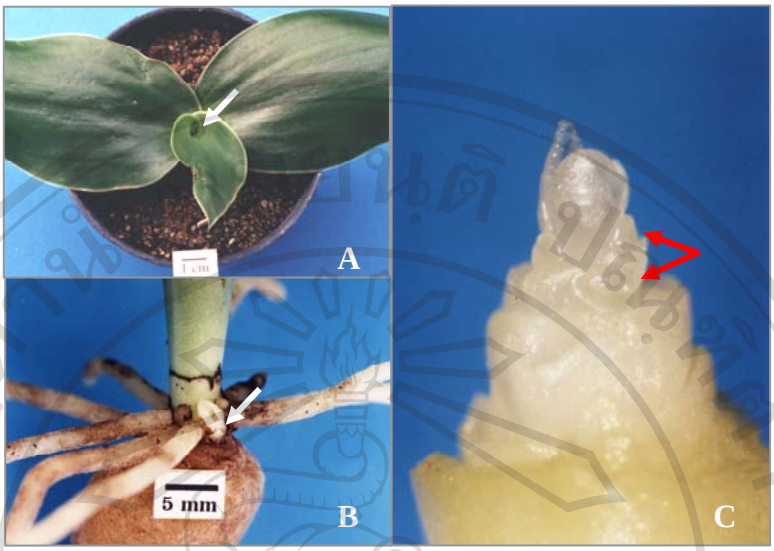


Figure 21 The reproductive stage of *Pecteilis sagarikii*.
A. Some leaf bracts were produced inside the central part of a mature plant (arrow)
B. A tuber primordium started to extend downwards (arrow)
C. The shoot meristem started to differentiate some young floret buds (arrows)

Figure 22 A new tuber produced a tuber-shoot bud (arrow) and continued to increase in the tuber size.

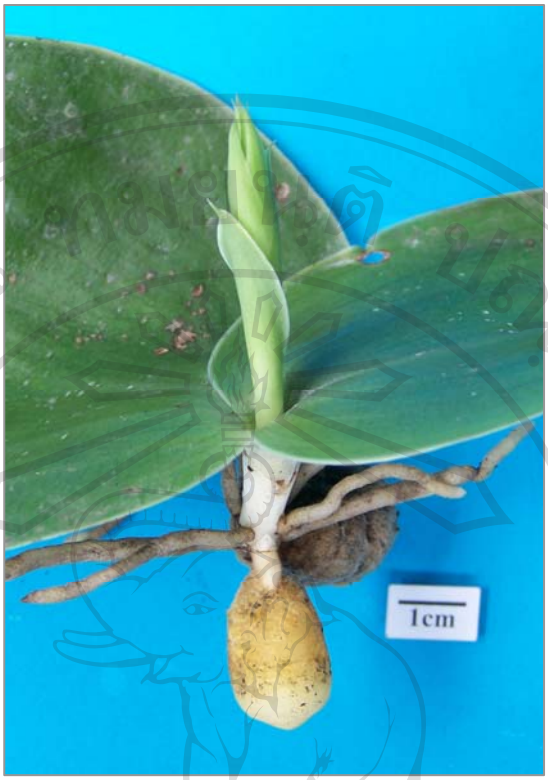


Figure 23 A young floral spike and a new tuber continued to increase in sizes.

Figure 24 A whole plant showing a complete new-formed tuber (arrow).

For the histological study on the morphological changes during *in vivo* tuberization of a mature plant observed under a light microscope, it showed that they could be divided into 4 stages as those of the *in vitro* tuberization as follows:

Stage 1 The formation of a tuber primordium
Stage 2 The extension of the tuber primordium
Stage 3 The formation of a tuber-shoot bud
Stage 4 The enlargement of the tuber

However, there were some differences in their details, especially in the stage 1 concerning the origins of the tuber primordia.

Stage 1 The formation of a tuber primordium

After shoot sprouting, plant continued to grow into the vegetative stage and produced 2 - 3 big expanded leaves. At the same time, the growing plant produced a group of meristematic cells which started cell division and produced some leaf primordia covering the meristem, and formed a complete adventitious bud at the lowest part of the plant monopodial stem which covered with a few dry leaf sheaths. At the end of the vegetative stage, the complete adventitious bud increased in size and produced new leaf primordia, and eventually formed a tuber primordium (Figure 25).

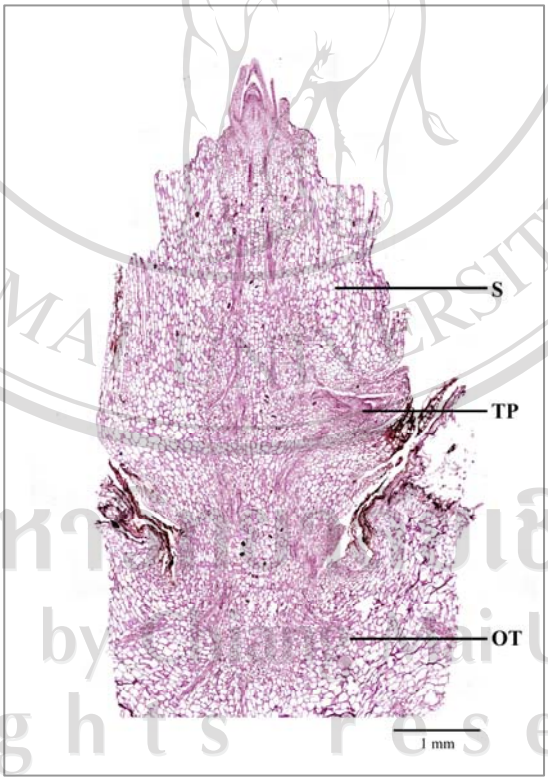


Figure 25 A longitudinal section of a vegetative shoot showing a tuber primordial bud originated at its lowest part of the plant monopodial stem.
S = Shoot, TP = Tuber primordium, OT = Old tuber

Stage 2 The extension of the tuber primordium

After the tuber primordium had been completely developed, the meristematic cells of the tuber primordium continued to divide and then grew to push the tuber primordium to the outward direction of the mother plant monopodial stem (Figure 26 A). After that, the lower part of the meristematic cells in the tuber primordium continued to divide and grew into the geotropic direction, together with the elongation of the cells at the base of the produced leaf primordia, pushing the tuber primordium extended straight down. Thereafter, a narrow hollow above the shoot meristem of the tuber primordium was formed (Figure 26 B and C). The tuber primordium continued to grow downwards to produce a tuber stalk until it reached an optimal depth.

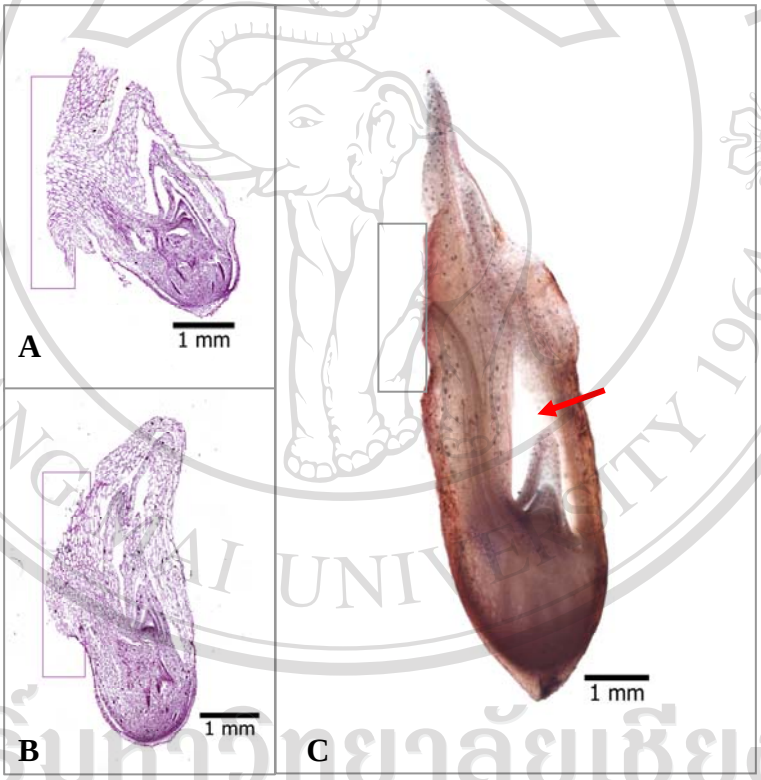


Figure 26 A longitudinal section of a tuber primordium showing the extension of a tuber primordial meristem and leaf primordia.
□ = Basal part of the mother plant
A. A tuber primordium started to grow and pushing itself from the mother plant
B. A tuber primordium started to grow in geotropic direction
C. A freezing microtome-cut tuber primordium formed a narrow hollow (arrow)

Stage 3 The formation of a tuber-shoot bud

While the tuber primordium reached almost an optimal depth, the lower meristematic cells in the tuber primordium had increasingly divided and started to separate far from their apical meristem. At this stage, the tuber primordium apparently had two groups of meristem, i.e. the apical meristem and a new lower meristem. When the tuber primordium moved downwards to reach an optimal depth, the apical meristem started to produce some leaf primordia to cover its meristem forming a new tuber-shoot bud for the next growing season (Figure 27). At this time the lower meristem continued to grow straight down forming a tuber tip.

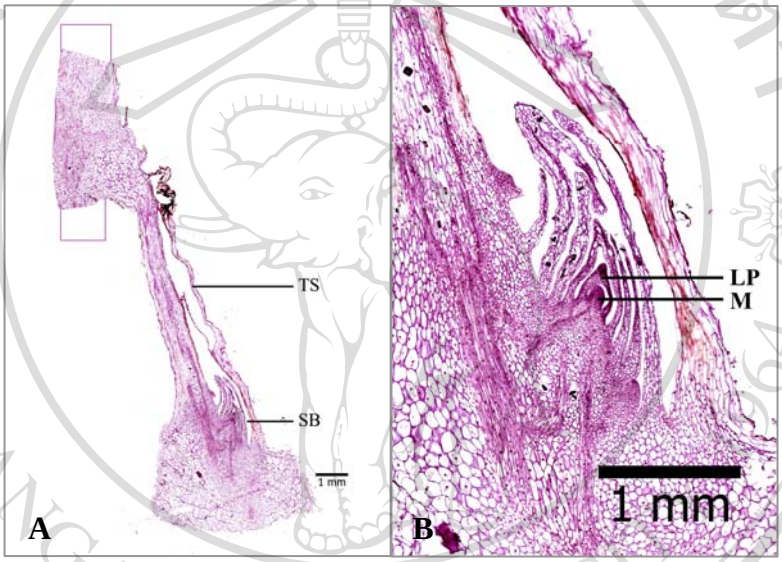


Figure 27 A longitudinal section of a new tuber showing the formation of a new tuber-shoot bud.
□ = Basal part of the mother plant, TS = Tuber stalk, SB = Shoot bud, LP = Leaf primordia, M = Meristem
A. A new tuber-shoot bud formed in the distal cavity at the base of the tuber stalk
B. A magnified new shoot bud showing a meristem covered with new leaf primordia

Stage 4 The enlargement of the tuber

In the final stage of the tuber development, the lower meristem showed cell division and continued to grow straight down to increase in tuber length, and to produce the vascular tissues which developed further into steles. At this stage, the cells surrounding the steles grew and enlarged in size resulting in increasing the tuber width. These processes continued until a complete tuber was obtained (Figure 28).

In addition, the tuber started to accumulate some starch granules in their cells, especially in the position near the steles and the meristematic cells, both in the new tuber-shoot bud and the tuber tip.

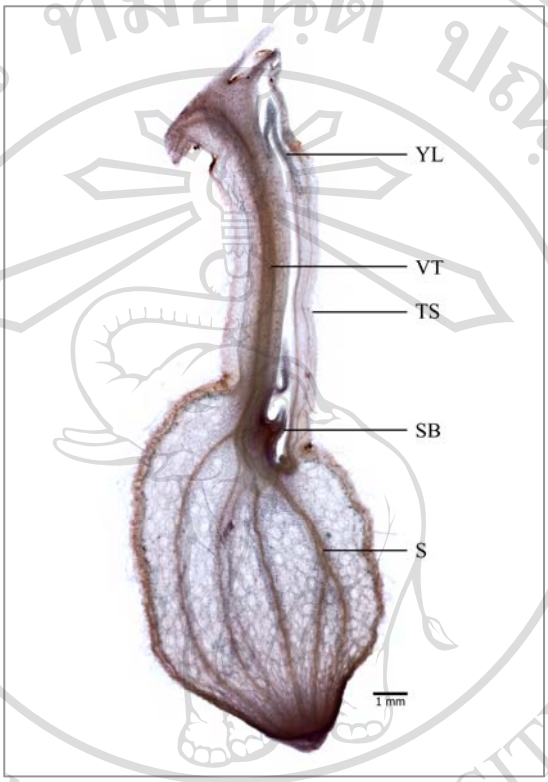


Figure 28 A longitudinal section of a new tuber using a freezing microtome.
YL = Young leaf, VT = Vascular tissue,
TS = Tuber stalk, SB = Shoot bud, S = Steles

Part III Studies on the changing of internal macro elements, free amino acids and some growth regulators in mature plants during tuberization.

1. *Fresh weight, dry weight and water contents*

The tubers showed increasing in fresh weight (FW) and dry weight (DW) at the shoot sprouting time, but had no obvious change in their water contents (WC). After that, the tuber lost in fresh and dry weights when grew and developed into the vegetative stage producing both shoot and root. The growing plant also decreased in total dry weight while it showed the increases in total fresh weight and the water content in all plant parts. In the pre-flowering stage, the plant showed increasing in fresh and dry weights in all plant parts, and also increased in total fresh and dry weights. But there was little change in their water contents percentage (Table 54).

Table 54 Changing of fresh weight (FW), dry weight (DW) and water content percentage (WC) in tuber, shoot and root in different growing stages of *Pecteilis sagarikii* grown in a green house.

		Dormancy stage	Early sprouting stage	Vegetative stage	Pre-flowering stage
FW (g)	Tuber	4.04±0.17	4.62±0.30	2.65±0.19	2.88±0.45
	Shoot	-	-	4.10±0.41	7.30±0.38
	Root	-	-	0.18±0.05	0.45±0.08
Total				*6.93*	*10.63*
DW (g)	Tuber	0.51±0.08	0.60±0.03	0.19±0.01	0.22±0.01
	Shoot	-	-	0.22±0.02	0.38±0.01
	Root	-	-	0.02±0.00	0.04±0.00
Total				*0.43*	*0.64*
WC (%)	Tuber	87.20±1.50	87.15±1.00	92.69±0.63	92.04±0.75
	Shoot	-	-	94.61±0.21	94.75±0.18
	Root	-	-	90.39±1.44	91.58±1.23

Data were averaged from 4 replicates, containing 6 plants/replicate.
- This organ was absent in these stages.

2. *Analysis of macro elements*

The concentration of nitrogen in the tuber at its dormancy stage was 11.19 mg/g DW and slightly increased when the tuber sprouted, and also increased more when the plant grew into the vegetative stage, and then slightly decreased at the pre-flowering stage. The nitrogen concentration in shoot at the vegetative stage was 20.85 mg/g DW, and then decreased in the pre-flowering stage (Table 55).

For the phosphorus, it increased in concentration in the tuber at sprouting stage and then slightly decreased in the vegetative stage and decreased more in the pre-flowering stage. Similarly, the decreases were also found in the shoot in the vegetative stage to the pre-flowering stage (Table 55).

The concentration of potassium in a tuber at different stages showed a similar change as the nitrogen concentration. It increased from the dormancy to the vegetative stage, and then slightly decreased at the pre-flowering stage. The changing of potassium concentration in the shoot was, nevertheless, slightly increased from the vegetative to the pre-flowering stage (Table 55).

Changing of calcium element, the concentration of calcium in the tuber had almost no change from dormancy to an early sprouting stage, but increased about two times in the vegetative stage. It then slightly decreased in the pre-flowering stage, the same as the changing of calcium concentration in the shoot that slightly decreased from the vegetative to the pre-flowering stage (Table 55).

As for magnesium element, it was found that the concentration of magnesium in the tuber slightly increased after the plant was in the vegetative stage and also the pre-flowering stage. But the magnesium concentration in the shoot from the vegetative period to the pre-flowering stage remained unchanged (Table 55).

Table 55 Concentrations of nitrogen, phosphorus, potassium, calcium and magnesium in a tuber and shoot in different growing stages of *Pecteilis sagarikii* grown in a green house.

Macro elements	Organs	Concentration of macro elements (mg/g DW)			
		Dormancy stage	Early sprouting stage	Vegetative stage	Pre-flowering stage
N	Tuber	11.19±1.61	12.21±1.58	16.16±3.21	15.29±2.13
	Shoot	-	-	20.85±1.10	18.74±2.07
P	Tuber	9.43±0.88	9.60±0.73	9.56±0.73	8.29±1.70
	Shoot	-	-	10.57±0.90	9.96±0.80
K	Tuber	1.06±0.15	1.10±0.07	1.65±0.14	1.56±0.18
	Shoot	-	-	2.31±0.06	2.48±0.12
Ca	Tuber	0.13±0.01	0.13±0.02	0.26±0.02	0.25±0.03
	Shoot	-	-	0.39±0.03	0.38±0.02
Mg	Tuber	0.10±0.02	0.10±0.02	0.14±0.01	0.16±0.02
	Shoot	-	-	0.19±0.01	0.19±0.01

3. *Analysis of free amino acid contents*

The analysis of free amino acids in the tuber showed that asparagine was the dominant free amino acid in all stages from dormancy to the pre-flowering stage. It started at 7,170.4 µgN/gDW in the dormancy stage and slightly decreased to 6,855.2 µgN/gDW in the tuber sprouting stage, and then obviously increased to 7,646.8 µgN/gDW in the vegetative stage, and finally decreased again to 6,588.3 µgN/gDW in the pre-flowering stage. In addition, the second abundant free amino acids were anserine and arginine. They have fluctuated in their concentrations in different stages of development (Table 56).

Table 56 Concentrations of free amino acids and distribution in *Pecteilis sagarikii* tuber in different growing stages.

Free amino acid		Dormancy stage		Early sprouting stage		Vegetative stage		Pre-flowering stage	
Name	Abbreviation	μgN/gDW	%	μgN/gDW	%	μgN/gDW	%	μgN/gDW	%
o-Phosphoethanolamine	P-EA	8.3±3.1	0.08	5.8±8.8	0.06	12.1±9.6	0.11	5.1±3.5	0.05
Aspartic acid	Asp	56.1±16.7	0.57	61.5±16.2	0.59	58.5±4.6	0.55	41.9±21.7	0.42
Threonine	Thr	3.9±1.9	0.04	4.4±5.3	0.04	7.4±3.9	0.07	8.3±5.5	0.08
Serine	Ser	53.0±52.7	0.54	52.3±100.2	0.50	34.5±22.5	0.32	68.8±51.9	0.69
Asparagine	Asn	7170.4±1699.4	72.71	6855.2±1218.7	65.61	7646.8±2252.6	71.51	6588.3±2578.9	66.06
Glutamine	Gln	22.5±16.6	0.23	73.1±109.1	0.70	24.2±29.6	0.23	68.2±75.4	0.68
α-Aminoadipic acid	A-AAA	0±0	0	16.1±32.2	0.15	13.5±15.6	0.13	25.4±30.8	0.25
Glycine	Gly	31.6±8.8	0.32	30.3±24.3	0.29	36.9±14.3	0.35	79.4±75.0	0.80
Alanine	Ala	96.8±22.8	0.98	72.6±54.2	0.69	54.9±15.7	0.51	91.8±51.8	0.92
α-Amino-n-butyric acid	A-ANBA	46.2±12.1	0.47	56.7±33.3	0.54	66.9±24.2	0.63	76.8±11.2	0.77
Valine	Val	1.9±3.8	0.02	5.9±7.2	0.06	0±0	0	0±0	0
Cystine	Cys	211.9±84.9	2.15	166.9±77.7	1.60	107.4±61.8	1.59	177.2±68.2	1.78
Isoleucine	Ileu	74.5±32.7	0.76	69.5±17.4	0.67	86.7±50.8	0.81	107.2±42.4	1.07
Leucine	Leu	51.8±39.8	0.52	32.0±19.1	0.31	45.0±20.3	0.42	67.8±24.7	0.68
Tyrosine	Tyr	9.2±18.4	0.09	6.5±13.0	0.06	0±0	0	0±0	0
Phenylalanine	Phe	16.6±6.8	0.17	11.4±4.2	0.11	18.0±10.3	0.17	22.9±8.2	0.23
β-Alanine	β-Ala	5.0±2.8	0.05	4.0±3.3	0.04	2.1±1.4	0.02	4.0±1.6	0.04
γ-Aminobutyric acid	GABA	9.2±5.3	0.09	12.8±11.6	0.12	0±0	0	38.2±31.1	0.38
Ethanolamine	EA	14.5±1.0	0.15	36.0±57.2	0.34	26.7±4.6	0.25	40.8±22.0	0.41
δ-Hydroxylysine	Hylys	207.5±90.8	2.10	335.1±177.5	3.21	371.3±192.3	3.47	526.6±72.3	5.28
Lysine	Lys	99.1±30.4	1.00	100.9±50.2	0.97	53.8±38.7	0.50	20.7±41.4	0.21
Histidine	His	122.9±31.2	1.25	118.1±37.5	1.13	92.4±67.9	0.86	58.7±69.3	0.59
3-Methylhistidine	3M-His	137.9±168.3	1.40	405.9±270.8	3.88	0±0	0	0±0	0
Anserine	Ans	434.0±250.3	4.40	347.6±104.3	3.33	1106.7±591.4	10.35	1365.1±698.5	13.69
Arginine	Arg	977.1±96.2	9.91	1568.5±1139.7	15.01	763.9±294.9	7.14	490.1±301.9	4.91
Total		***9861.9***	***100***	***10448.8***	***100***	***10692.6***	***100***	***9973.3***	***100***

In the plant shoot, it still showed that the asparagine was the dominant free amino acid in both the vegetative and pre-flowering stages. It had 2,751.8 µgN/gDW or 68.95 % of the total free amino acids in the vegetative stage and then decreased in the following pre-flowering stage. In addition, it was found that anserine was the second abundant free amino acid in the vegetative stage and decreased about a half in the pre-flowering stage (Table 57).

Table 57 Concentrations of free amino acids and distribution in *Pecteilis sagarikii* shoot in different growing stages.

Free amino acid		Vegetative stage		Pre-flowering stage	
Name	Abbreviation	µgN/gDW	%	µgN/gDW	%
o-Phosphoethanolamine	P-EA	2.6±1.6	0.07	1.0±0.8	0.03
Aspartic acid	Asp	19.7±10.8	0.49	17.2±5.4	0.50
Threonine	Thr	2.6±1.8	0.07	2.1±0.7	0.06
Serine	Ser	26.2±18.9	0.66	25.5±15.1	0.74
Asparagine	Asn	2751.8±906.9	68.95	2163.6±659.1	62.79
Glutamine	Gln	43.2±6.4	1.08	54.6±30.9	1.59
α-Aminoadipic acid	A-AAA	0±0	0	11.1±8.4	0.32
Glycine	Gly	82.0±13.7	2.06	64.5±48.9	1.87
Alanine	Ala	44.8±14.2	1.12	49.2±15.0	1.43
α-Amino-n-butyric acid	A-ANBA	33.5±18.5	0.84	28.5±18.5	0.83
Valine	Val	1.2±2.3	0.03	0±0	0
Cystine	Cys	101.4±24.2	2.54	65.6±39.4	1.90
Isoleucine	Ileu	41.1±6.0	1.03	33.6±13.8	0.98
Leucine	Leu	20.2±3.8	0.51	21.6±10.4	0.63
Tyrosine	Tyr	0±0	0	142.7±285.4	4.14
Phenylalanine	Phe	4.9±1.4	0.12	2.6±2.6	0.08
β-Alanine	β-Ala	0.4±0.8	0.01	0.5±0.6	0.01
γ-Aminobutyric acid	GABA	31.6±21.5	0.79	36.0±15.9	1.05
Ethanolamine	EA	29.4±1.7	0.74	17.5±10.8	0.51
δ-Hydroxylysine	Hylys	173.5±36.8	4.35	172.3±84.0	5.00
Lysine	Lys	0±0	0	25.7±24.9	0.75
Histidine	His	21.6±9.4	0.54	129.0±108.5	3.74
3-Methylhistidine	3M-His	0±0	0	0±0	0
Anserine	Ans	399.5±162.0	10.01	174.6±96.0	5.07
Arginine	Arg	159.5±104.0	4.0	206.5±326.3	5.99
Total		***3990.7***	***100***	***3445.7***	***100***

In root, asparagine was the dominant free amino acid both in the vegetative and pre-flowering stages. It had 6,837.2 µgN/gDW in the vegetative stage and increased to 7,147.9 µgN/gDW in the pre-flowering stage. In addition, arginine and anserine were the second and third abundant free amino acids in roots in the vegetative stage at 3,070.7 and 790.0 µgN/gDW, respectively, and then markedly decreased in their concentrations in the pre-flowering stage (Table 58).

Table 58 Concentrations of free amino acids and distribution in *Pecteilis sagarikii* root in different growing stages.

Free amino acid		Vegetative stage		Pre-flowering stage	
Name	Abbreviation	µgN/gDW	%	µgN/gDW	%
o-Phosphoethanolamine	P-EA	19.1±1.8	0.15	13.1±16.0	0.12
Aspartic acid	Asp	63.8±13.9	0.52	53.4±43.8	0.48
Threonine	Thr	6.8±0.4	0.05	2.6±3.7	0.02
Serine	Ser	96.9±28.2	0.78	44.5±69.1	0.40
Asparagine	Asn	6837.2±1120.0	55.24	7147.9±3921.8	64.23
Glutamine	Gln	174.2±13.7	1.41	162.2±160.3	1.46
α-Aminoadipic acid	A-AAA	0±0	0	17.1±34.2	0.15
Glycine	Gly	38.3±5.1	0.31	30.7±21.9	0.28
Alanine	Ala	107.8±40.1	0.87	83.8±57.9	0.75
α-Amino-n-butyric acid	A-ANBA	59.5±23.9	0.48	64.2±53.0	0.58
Valine	Val	5.4±10.9	0.04	1.6±3.3	0.01
Cystine	Cys	174.2±70.3	1.41	164.6±131.5	1.48
Isoleucine	Ileu	97.5±6.6	0.79	74.7±31.7	0.67
Leucine	Leu	52.8±6.9	0.43	48.0±23.1	0.43
Tyrosine	Tyr	0±0	0	0±0	0
Phenylalanine	Phe	16.8±0.9	0.14	7.7±2.3	0.07
β-Alanine	β-Ala	6.0±0.3	0.05	2.4±1.8	0.02
γ-Aminobutyric acid	GABA	31.2±6.8	0.25	23.2±6.8	0.21
Ethanolamine	EA	119.8±17.2	0.97	99.0±64.1	0.89
δ-Hydroxylysine	Hylys	331.9±38.6	2.68	347.2±180.3	3.12
Lysine	Lys	107.5±47.4	0.87	157.2±145.7	1.41
Histidine	His	106.6±57.4	0.86	60.5±24.5	0.54
3-Methylhistidine	3M-His	63.5±127.0	0.51	0±0	0
Anserine	Ans	790.0±371.4	6.38	606.3±403.1	5.45
Arginine	Arg	3070.7±708.0	24.81	1916.9±1382.6	17.22
Total		***12377.6***	***100***	***11128.8***	***100***

4. *Analysis of free indoleacetic acid (IAA) and abscisic acid (ABA) contents*

The analysis of free IAA and ABA in both tuber and shoot at different stages showed that the tuber samples were not successfully extracted, purified and analyzed by the used protocol (Appendix C-11); after injecting the purified sample into the Gas Chromatography Mass Spectrophotometer (GCMS), the peaks of both ^{13}C-IAA and ^{2}H-ABA, which were used as the standard hormones could not be detected. Similarly, the peaks of IAA and ABA of the tuber sample also could not be seen. It should be mentioned that although the extraction protocol was adjusted in some details, e.g. the concentration and the volume of phosphate-Na buffer solution, the peak detection was also unsuccessful.

For the shoot, the free IAA concentration was about 1,027 ng/gDW at the vegetative stage and obviously decreased to 300 ng/gDW at the pre-flowering stage, in the same trend as the concentration of free ABA in the vegetative stage, i.e. about 2,085 ng/gDW and decreased to 1,439 ng/gDW in the pre-flowering stage (Table 59).

Table 59 Concentrations of free indole-3-acetic acid (IAA), abscisic acid (ABA), and their distributions in *Pecteilis sagarikii* tuber and shoot in different growing stages.

Growth regulator	Organ	Concentration (ng/gDW)			
		Dormancy stage	Early sprouting stage	Vegetative stage	Pre-flowering stage
IAA	Tuber	*	*	*	*
	Shoot	-	-	1,027±829	300±90
ABA	Tuber	*	*	*	*
	Shoot	-	-	2,085±694	1,439±1,055

Data were averaged from 3 replicates, containing 6 plants/replicate.

* The samples were not successfully extracted, purified and analyzed.

\- This organ was absent in these stages.