MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

LE THE BIEN

IN VITRO MORPHOGENESIS AND GROWTH OF SOME ECONOMICALLY VALUABLE PLANT SPECIES UNDER SIMULATED MICROGRAVITY CONDITION

SUMMARY OF DISSERTATION ON BIOTECHNOLOGY

Code: 9 42 02 01

Hanoi - 2024

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science and Technology.

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The dissertation was examined by the Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at 8:30, April 06, 2024.

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INTRODUCTION

The necessity of the dissertation

Gravity is a constant quantity that plays a decisive role in the survival, growth, development and evolution of plants. Therefore, the question is "when plants escape the influence of gravity, what effects will they have?" Can they develop their amazing flexibility to cope with greatly changed living conditions as they have encountered in their evolutionary history? That is the question that has challenged scientists for a very long time.

Over the past five decades, along with the explosive development of space science, exciting new insights into plant life in altered gravity environments have gradually been revealed. As humans explore deep into space, the logistics and economics of transporting packaged food to crew members become increasingly inadequate. Besides, nutrients in packaged foods tend to lose quality significantly when stored in space. Therefore, plant biology experiments in space are necessary to provide fresh food for astronauts and create a foundation for building a sustainable life support biological regeneration system for humans to explore space for a long time.

Biological experiments in altered gravity conditions have been performed through orbital spacecraft flights and Simulated microgravity (SMG) platforms using instruments such as Clinostat (2-D and 3-D), Random positioning machine (RPM) on the ground.For example, the effects of microgravity (MG) on changes at the cellular and molecular levels that lead to altered plant phenotypes such as changes in the cell wall and cell cycle have been elucidated in detail. Vigorous division and proliferation of plant cells were clearly observed under MG conditions, and physiological responses, especially the distribution/translocation of statolith cells,

photosynthesis, and deformation of starch granules have been reported. Specific biochemical changes in chlorophyll content, plant hormone localization, and cellular calcium homeostasis in response to microgravity have been well documented. The knowledge gained through these experiments has been successfully applied to the cultivation of Lettuce plants by NASA on the ISS to make a useful contribution to the crew's diet.

In addition, results from the Space Seeds for the Future Asia Program for the period 2010 - 2011 show that processing seeds under MG and then planting them on the ground has shown that the seeds stimulate more germination and increase the ability to accumulate secondary compounds in the plant. This opens up great potential for the field of biotechnology in selecting and creating new varieties as well as producing biomass and obtaining valuable medicinal substances for the pharmaceutical industry.

However, most of the above research is conducted in space and requires high technical expertise from scientists, strict control regimes, and modern facilities, so it is limited within the range of several space powers. Developing countries like Vietnam do not have enough economic potential and scientific and technical levels to conduct research under these conditions. Therefore, the choice of a more feasible solution was motivated by a ground-based microgravity simulator considered suitable for active and long-term research in the context of space science still very young in the country. In particular, research to understand the process of plant morphology and growth using tissue culture under SMG conditions is the optimal method for recording the role of MG at early stages in the plant life cycle. From there, selecting new mutations provides raw materials for plant breeding and biomass production, as well as

providing new knowledge for humanity's mission of conquering space by creating biological life support systems beyond Earth.

The thesis "*In vitro* morphogenesis and growth of some economically valuable plant species under simulated microgravity condition" is a novelty research direction in the world as well as in Vietnam. The research was carried out on three types of plants representing ornamental flowers (*Begonia*), fruit (Strawberry), and medicinal plants (*Phyllanthus*) which have been confirmed to have high economic value as well as the expectation that they are crop varieties capable of meeting the basic nutritional, health and spiritual needs of humans living outside the Earth.

Objective and requirement of the dissertation

General objective

Evaluate the effects of SMG conditions on the *in vitro* morphogenesis and growth of *Begonia*, Strawberry, and *Phyllanthus* plants.

Detail objective

Evaluate the effects of SMG conditions on the *in vitro* morphological pathways of *Begonia*, Strawberry and *Phyllanthus* plants.

Evaluate the effects of SMG conditions on the content and ratio of endogenous hormones, antioxidant enzyme activity, secondary compound accumulation and energy metabolism during the *in vitro* morphogenesis of *Begonia*, Strawberry, and *Phyllanthus* plants.

Evaluate the growth under gravity of *Begonia*, Strawberry, and *Phyllanthus* plants which derived from SMG conditions.

Chapter I: LITERATURE REVIEW

Ph.D. thesis has been referred to use in this dissertation related to: (1) Plant and gravity; (2) Microgravity and MG; (3) Morphogenesis

under MG; (4) Plant growth under MG; (5) Physiology – biochemistry of plant under MG; (6) General of subjects.

Chapter II: MATERIALS, CONTENTS AND METHODS

2.1. Materials

2.1.1. Plant materials

The *ex vitro* petiole of 4-month-old *Begonia tuberous* (Dalat Hasfarm, Lam Dong, Vietnam) were used as the initial explant source. The 4-week-old *in vitro* leaves of Strawberry Camarosa (*Fragaria* × *ananassa* Duch.) have been determined for genetic diversity and have been stabilized through multiple subcultures on MS medium at the Centre for Biodiversity and Climate change research (Da Lat University, Lam Dong, Vietnam) was used as the initial explant source. The *in vitro* internode of 4-week-old *Phyllanthus amarus* Schum. & Thonn. were stabilized through many times of subcultures on MS medium at the Tay Nguyen Institute for Scientific Research (Lam Dong, Vietnam) was used as the initial explant source.

2.1.2. Culture media

The medium used for the experiments was MS medium supplemented with/whitout plant growth regulators depending on the purpose of the experiment. The pH is adjusted to 5.8 and sterilized in an autoclave at 121°C, 15 psi for 20 min.

2.1.3. Equipment, instruments and chemicals

The Clinostat 2-D (Advanced Engineering Services Co., Ltd., Japan) with a rotation speed of 2 rpm was selected for SMG purposes. This was the slow (classical) rotation speed used by plant physiologists for research. Instruments and chemicals used in routine cell tissue culture.

2.2. Research contents

Content 1: Studying the effects of SMG conditions on the *in vitro* morphogenetic pathways of *Begonia*, Strawberry, and *Phyllanthus* plants.

Content 2: Studying the effects of SMG conditions on the content and ratio of endogenous hormones, antioxidant enzyme activity, secondary compound accumulation and energy metabolism during the *in vitro* morphogenesis of *Begonia*, Strawberry, and *Phyllanthus* plants.

Content 3: Studying the growth under gravity of *Begonia*, Strawberry, and *Phyllanthus* plants which derived from SMG conditions.

2.3. Research methods

2.3.1. Design of experiments

2.2.1.1. Experiment 1: Studying the effects of SMG conditions on somatic embryogenesis (SEs) of *Begonia* p-tTCL cultured *in vitro*

2.2.1.2. Experiment 2: Studying the effects of SMG conditions on callus induction and shoot regeneration of Strawberry leaf cultured *in vitro*

2.2.1.3. Experiment 3: Studying the effects of SMG conditions on the callus induction and adventitious root formation of *Phyllanthus* internode cultured *in vitro*

2.2.1.4. Experiment 4: Studying the effects of SMG conditions on content and ratio of endogenous hormones in *Begonia* SEs, Strawberry shoot, *Phyllanthus* callus and adventitious root cultured *in vitro*

2.2.1.5. Experiment 5: Studying the effects of SMG conditions on antioxidant enzyme activity in Strawberry callus, *Phyllanthus* callus and adventitious root cultured *in vitro*

2.2.1.6. Experiment 6: Studying the effects of SMG conditions on secondary compound accumulation in *Phyllanthus* callus and adventitious root cultured *in vitro*

2.2.1.7. Experiment 7: Studying the effects of SMG conditions on energy metabolism (starch and sugar) in *Begonia* SEs cultured *in vitro*

2.2.1.8. Experiment 8: Studying the growth of shoot or plantlet derived from SMG conditions under gravity

2.2.1.9. Experiment 9: Studying the proliferation of *Phyllanthus* callus derived from SMG conditions under gravity

2.2.1.10. Experiment 10: Studying the acclimatization of Strawberry plantlet derived from SMG conditions in the greenhouse

2.3.2. Plant anatomy

2.3.3. Data analysis

Chapter III: RESULTS AND DISCUSSION

3.1. Effects of SMG conditions on SEs of *Begonia* p-tTCL cultured *in vitro*

The results showed that SMG conditions did not affect the regeneration of the explants with regeneration reaching 100% in SMG and G treatments after 30 days of culture (Fig. 3.1A-D). However, on the 14th day, the explants under SMG treatment began to show SEs (Fig. 3.1B); While none of the epidermal cells under G treatment showed this induction (Fig. 3.1A). Furthermore, morphological observations showed that SEs from embryogenic callus under G condition (Fig. 3.1C) occurred at a slower rate than under SMG condition (Fig. 3.1D) after 30 days of culture. It can be seen that the SMG condition shortened the induction time for SEs compared to the G condition (about 5 days) (Fig. 3.1E). After 3 months of culture, the results showed that two differentiation

programs took place in both SMG and G conditions: SEs (Fig. 3.2A-C) and adventitious roots (Fig. 3.2.D). In particular, SEs is predominant while adventitious roots were only formed in negligible quantities (data not shown). These results indicated that the SMG condition did not interfere with the differentiation of Begonia p-tTCL. Furthermore, observation of plant anatomy showed that under SMG conditions, SEs still undergoes typical developmental stages of somatic embryos similar to G (Fig. 3.2E). The effects of SMG conditions on the growth of SEs were evaluated through fresh weight, dry weight, and chlorophyll content (Fig. 3.3). The morphological observations showed that stomata under G treatment have an oval shape (Fig. 3.4.A); whereas the stomata under SMG treatment were elliptical (elongated) and the larger stomatal opening was observed (Fig. 3.4.B). Under SMG treatment, stomata were 1.4 times longer than the control; Meanwhile, the width of the stomata did not change under SMG conditions (Fig. 3.4.C).

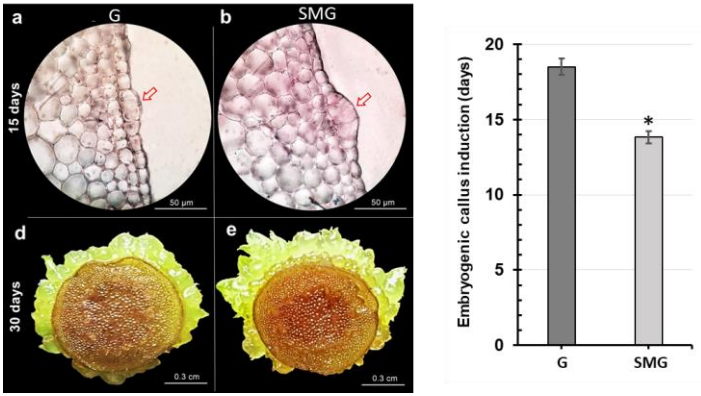

Figure 3.1. Efect of SMG conditions on SEs of *Begonia* p-tTCL cultured *in vitro* after 30 days. **A, B:** SEs starting from the epidermis of p-tTCL after 15 days (arrow indicates cell induction); **C, D:** SEs differentiation from embryogenic callus after 30 days; **E:** SEs induction time; *Significant difference between mean according to Tukey test ($p < 0.05$). Images were observed under an optical microscope at a ×40 objective.

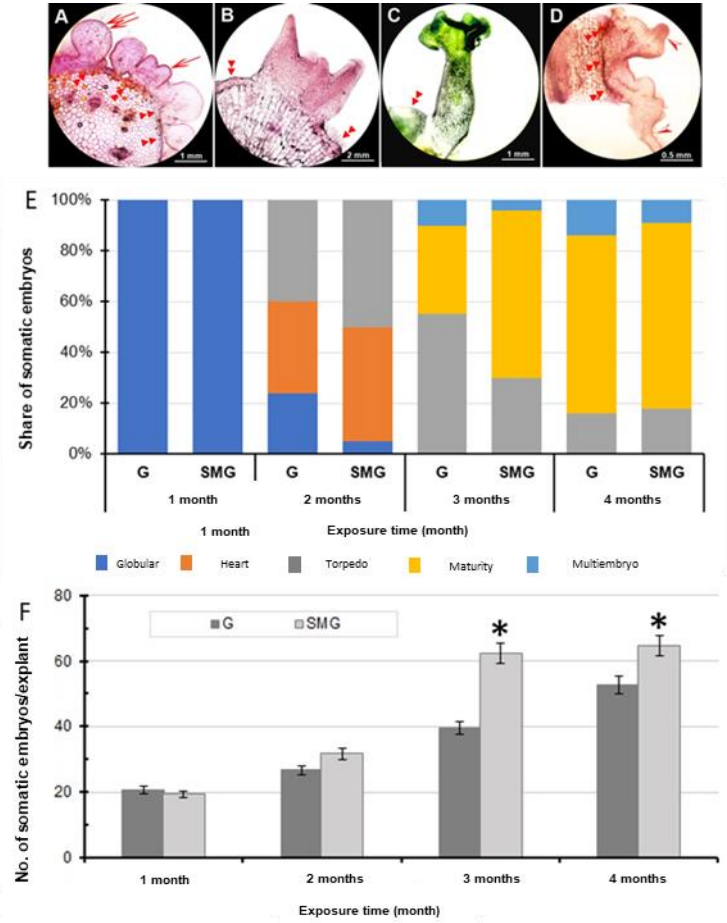

Figure 3.2. Effect of SMG conditions on the SEs stages of *Begonia* p-tTCL cultured *in vitro* after 1, 2, 3 and 4 months. **A-C:** SE stages observed under an optical microscope at a ×40 objective, double triangle indicates the epidermis of p-tTCL explant; **A:** Spherical embryos (double arrow) and heart-shaped embryos (arrow) after 2 months of culture; **B:** Multiple embryos after 3 months; **C:** Torpedo-shaped embryo after 2 months; **D:** Adventitious roots (arrows) from the epidermis of p-tTCL after 2 months**; E:** The percentage of SEs shapes; **F:** Total number of SEs at the time of recording; *Significant difference between mean according to Tukey test ($p < 0.05$).

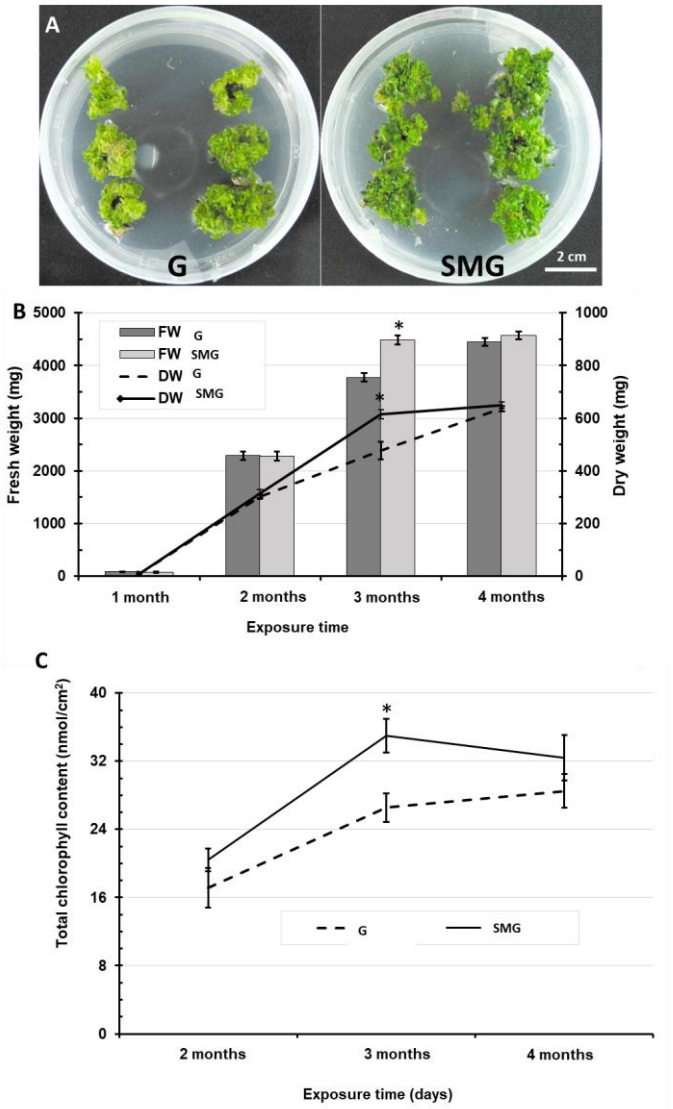

Figure 3.3. Effect of SMG conditions on the SEs development in *Begonia* p-tTCL cultured *in vitro* after 1, 2, 3 and 4 months. **A:** SEs under SMG and G conditions after 3 months; **B:** Fresh weight and dry weight of SEs; C: Chlorophyll content in leaves of mature SEs; *Significant difference between mean according to Tukey test ($p < 0.05$).

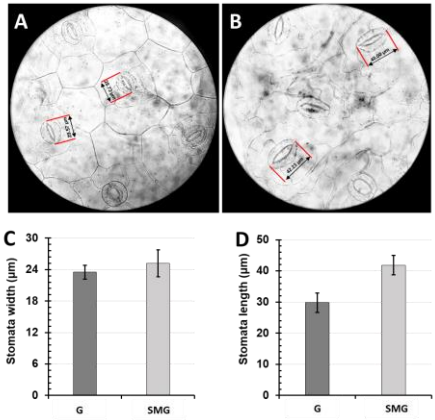

Figure 3.4. Effect of SMG conditions on the stomata morphology in SEs mature leaf of Begonia p-tTCL cultured *in vitro*. **A:** G conditions; **B:** SMG conditions; **C:** Length and width of stomata; *Significant difference between mean according to Tukey test ($p < 0.05$). Stomata were observed under an optical microscope at a ×40 objective.

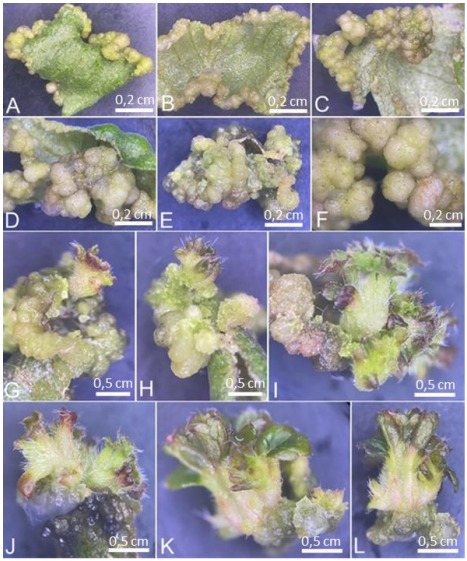

Figure 3.5. Effect of SMG conditions on the callus induction and shoot regeneration of Strawberry *in vitro* leaf after 2, 3, 4 and 6 weeks. Callus induction after 2 weeks **(A)**, 3 weeks (**B, C**), 4 weeks (**D**), 6 weeks (**E, F**; Shoot regeneration after 3 weeks (**G**) under SMG; Shoot regeneration after 4 weeks under G (**H**) and SMG (**I**); Shoot regeneration after 6 weeks under (**J**) and SMG (**K, L**).

3.2. Effects of SMG conditions on callus induction and shoot regeneration of Strawberry leaf cultured *in vitro*

The results showed that the callus induction time (2 weeks) and callus morphology under SMG and G treatments were observed to be similar; However, the shoot regeneration of *in vitro* leaf under the two conditions was different (Table 3.1 and Fig. 3.5).

Table 3.1. Effect of SMG conditions on the callus induction and shoot regeneration of Strawberry *in vitro* leaf after 2, 3, 4 and 6 weeks

Duration	**2 weeks**		**3 weeks**		**4 weeks**		**6 weeks**	
Characters	**G**	**SMG**	**G**	**SMG**	**G**	**SMG**	**G**	**SMG**
Callus induction (%)	25.00 ±2.89^{d*}	53.33 ±1.45^{bc}	46.33 ± 1.45^{c}	66.33 ± 1.45^{a}	62.00 ±2.89^{ab}	57.00 ±1.53^{abc}	62.67 ± 2.19^{ab}	56.67 ± 3.48^{abc}
Without callus induction (%)	75.00 ± 2.89^{a}	46.67 ± 1.45^{b}	53.67 ± 1.45^{b}	27.00 ± 1.53^{c}	25.00 ± 2.89^{c}	23.67 ± 0.67^{cd}	16.00 ± 1.00^{d}	7.00 ± 1.54^{e}
Shoot regeneration (%)	-**	-	-	6.67 ± 0.89^{d}	13.00 ± 0.00^{c}	19.33 ± 1.20^{b}	21.33 ± 1.76^{b}	36.33 ± 2.33^{a}
Fresh weight (mg)	90.86 ± 3.88^{e}	117.49 ± 2.18^{d}	114.34 ± 5.00^{d}	158.85 ± 4.47^{c}	149.69 ± 3.67^{c}	194.20 ± 4.51^{b}	183.25 ± 3.94^{b}	223.25 ± 6.58^{a}
Dry weight (mg)	10.92 ± 0.43^{e}	13.83 ± 0.30^{d}	13.61 ± 0.36^{d}	17.69 ± 0.48^{c}	17.65 ± 0.05^{c}	20.85 ± 0.85^{b}	19.48 ± 0.42^{bc}	24.97 ± 0.19^{a}

**Different letters (a, b) in the same row represent statistically significant differences ($p < 0.05$) between treatments according to Tukey's test. Data are mean ± SE (standard error); ** No data recorded.*

3.3. Effects of SMG conditions on the callus induction and adventitious root formation of *Phyllanthus* internode cultured *in vitro*

The results showed that there was no difference in the callus induction time of the Phyllanthus internode under SMG and G conditions. However, the rates of callus induction and adventitious root formation showed differences between the two culture conditions (Table 3.2).

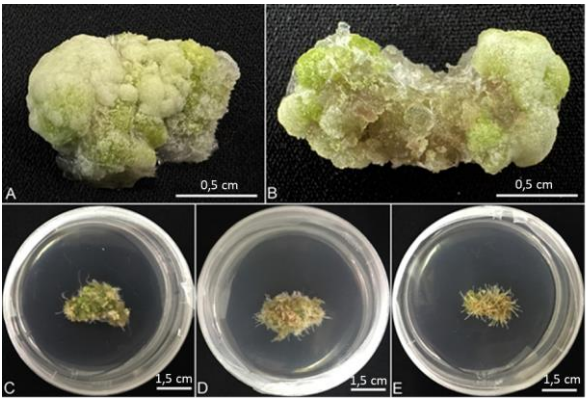

Figure 3.6. Effects of SMG conditions on the callus induction and adventitious root formation of *Phyllanthus* internode cultured *in vitro* after 2, 3 and 4 weeks. **A:** Callus under G after 4 weeks; **B:** Callus under SMG after 4 weeks; **C, D, E:** Adventitious root under SMG after 2, 3 and 4 weeks.

After 4 weeks of culture, the rate of callus induction under SMG conditions was only 34.33% and the rate of adventitious root formation increased to 65.67%. Under G conditions, without adventitious root formation was recorded after 4 weeks of culture (Table 3.2 and Fig. 3.6).

Table 3.2. Effects of SMG conditions on the callus induction and adventitious root formation of Phyllanthus internode cultured *in vitro* after 2, 3 and 4 weeks

Duration	**2 weeks**		**3 weeks**		**4 weeks**	
Characters	**G**	**SMG**	**G**	**SMG**	**G**	**SMG**
Callus induction (%)	100,00 ± 0,00^{a*}	90,33 ± 0,67^{b}	100,00 ± 0,00^{a}	62,67 ± 1,76^{b}	100,00 ± 0,00^{a}	34,33 ± 1,20^{c}
Adventitious root (%)	-**	6,67 ± 0,67^{c}	-	37,33 ± 1,76^{b}	-	65,67 ± 1,20^{a}
Fresh weight (mg)	263,00 ± 3,79^{f}	541,00 ± 3,21^{c}	291,00 ± 1,53^{e}	598,67 ± 4,33^{b}	313,33 ± 2,03^{d}	792,00 ± 1,73^{a}
Dry weight (mg)	24,67 ± 0,67^{e}	53,83 ± 0,41^{c}	29,00 ± 0,45^{d}	59,89 ± 0,43^{b}	30.07 ± 0,25^{d}	79,17 ± 0,27^{a}

**Different letters (a, b) in the same row represent statistically significant differences ($p < 0.05$) between treatments according to Tukey's test. Data are mean ± SE; ** No data recorded.*

3.4. Effects of SMG conditions on content and ratio of endogenous hormones in *Begonia* SE, Strawberry shoot, *Phyllanthus* callus and adventitious root cultured *in vitro*

For *Begonia*, the difference was significant in the SEs stages of the p-tTCL explants (Fig. 3.7).

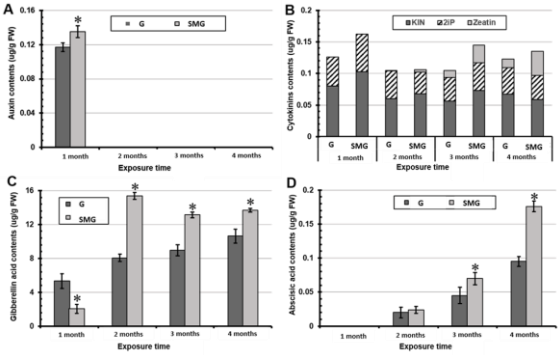

Figure 3.7. Effects of SMG conditions on content and ratio of endogenous hormones in SEs of *Begonia* p-tTCL cultured *in vitro*. **A:** AUX content; **B:** KIN, 2iP, ZEA contents; **C:** GA_3 content; **D:** ABA content; **Significant difference between mean according to Tukey test ($p < 0.05$).*

For Strawberry, shoots derived from SMG treatment recorded higher GA3, ABA, and KIN contents compared to those under G treatment after 6 weeks of culture; Meanwhile, AUX and ZEA contents showed the opposite result (Fig. 3.8). 2iP content has almost no difference in both culture conditions (Fig. 3.8).

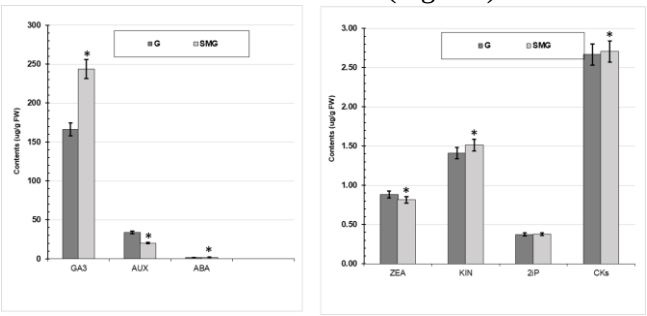

Figure 3.8. Effects of SMG conditions on content and ratio of endogenous hormones in shoot of Strawberry *in vitro* leaf after 6 weeks; **Significant difference between mean according to Tukey test ($p < 0.05$).*

For *Phyllanthus*, callus derived from SMG treatment obtained GA_3, AUX, and ABA content higher than those in G treatment; meanwhile, KIN content showed the opposite result (Table 3.3).

Table 3.3. Effects of SMG conditions on content and ratio of endogenous hormones in *Phyllanthus* callus and adventitious root cultured *in vitro* after 4 weeks

Treatment		GA_3	AUX	ABA	ZEA	KIN	2iP
G	Callus	28.00 ± 0.130^{b}	2.12 ± 0.017^{b}	0.88 ± 0.003^{c}	0.19 ± 0.006^{a}	0.23 ± 0.009^{a}	0.032 ± 0.001^{a}
SMG	Callus	29.62 ± 0.064^{a}	3.24 ± 0.052^{a}	1.81 ± 0.018^{a}	0.18 ± 0.006^{a}	0.17 ± 0.006^{b}	0.028 ± 0.001^{a}
	Adventitious root	27.62 ± 0.064^{b}	2.06 ± 0.009^{b}	1.74 ± 0.009^{b}	0.17 ± 0.001^{a}	0.17 ± 0.011^{b}	0.27 ± 0.001^{a}

**Different letters (a, b) in the same row represent statistically significant differences ($p < 0.05$) between treatments according to Tukey's test. Data are mean ± SE.*

3.5. Effects of SMG conditions on antioxidant enzyme activity in Strawberry callus, *Phyllanthus* callus and adventitious root cultured *in vitro*

For Strawberry, APX, CAT, DPPH and phenolic content had differences in both culture conditions after 2, 3, 4 and 6 weeks (Table 3.4). For *Phyllanthus*, SOD, APX, CAT and phenolic content recorded differences in SMG and G conditions after 2, 3 and 4 weeks of culture (Table 3.5).

Table 3.4. Effects of SMG conditions on antioxidant enzyme activity in Strawberry callus after 2, 3, 4 and 6 weeks of culture

Duration	2 weeks		3 weeks		4 weeks		6 weeks	
Characters	G	SMG	G	SMG	G	SMG	G	SMG
CAT (U/g)	96,82 ± 0,929^{f}	107,69 ±1,227^{e}	121,13 ±2,147^{d}	137,67 ± 0,633^{c}	140,05 ±2,887^{c}	156,73 ±0,829^{b}	163,42 ± 0,229^{ab}	167,90 ± 0,241^{a}
APX (U/g)	0,11 ± 0,006^{e}	0,16 ± 0,007^{bc}	0,18 ± 0,003^{b}	0,22 ±0,007^{a}	0,15 ± 0,003^{cd}	0,17 ± 0,003^{b}	0,12 ± 0,007^{de}	0,11 ± 0,006^{e}
DPPH (% RSA)	6,61 ± 0,208^{f}	8,20 ± 0,062^{de}	7,56 ± 0,026^{e}	8,69 ± 0,174^{d}	8,06± 0,050^{de}	11,42 ± 0,136^{b}	9,53 ± 0,136^{c}	13,38 ± 0,15^{a}
Phenolic (mg GAE /100 g Dry weight)	124,57 ± 2,378^{d}	81,84 ± 2,436^{f}	131,38 ±0,882^{d}	96,05 ± 1,443^{e}	171,72 ±0,667^{a}	148,69 ± 2,273^{c}	161,88 ± 1,167^{b}	155,38 ± 2,315^{bc}

**Different letters (a, b) in the same row represent statistically significant differences ($p < 0.05$) between treatments according to Tukey's test. Data are mean ± SE.*

Table 3.5. Effects of SMG conditions on antioxidant enzyme activity in *Phyllanthus* callus and adventitious root after 2, 3 and 4 weeks of culture

Duration	**2 weeks**			**3 weeks**			**4 weeks**		
	G	**SMG**		**G**	**SMG**		**G**	**SMG**	
Characters	**Callus**	**Callus**	Adventitious root	**Callus**	**Callus**	Adventitious root	**Callus**	**Callus**	Adventitious root
SOD (U/g)	22,28 ± 0,36^{i}	51,23 ±0,48^{f}	75,79 ± 0,20^{c}	31,18 ± 0,52^{h}	60,90 ± 0,61^{e}	90,42 ± 0,36^{b}	44,72 ± 0,71^{g}	71,38 ±0,83^{d}	121,39 ±0,41^{a}
CAT (U/g)	174,95 ± 1,37^{f}	181,05 ± 0,74^{f}	227,70 ± 0,70^{d}	215,81 ± 1,19^{e}	222,80 ± 1,5^{de}	303,70 ± 3,18^{b}	236,46 ± 1,77^{c}	243,29 ± 1,15^{c}	344,79 ± 1,21^{a}
APX (U/g)	0,28 ±0,012^{ef}	0,24 ± 0,003^{f}	0,63 ± 0,023^{b}	0,35 ±0,015^{de}	0,32 ± 0,012^{ef}	0,67 ±0,023^{b}	0,43± 0,023^{cd}	0,44 ±0,009^{c}	0,95± 0,015^{a}
Phenolic (mg GAE/100 g CK)	50,91 ± 0,607^{f}	56,47 ±0,364^{f}	81,40 ±0,549^{d}	71,56 ± 0,820^{e}	77,19 ± 1,025^{de}	97,33± 0,884^{bc}	92,31 ±0,787^{c}	99,24 ±0,549^{b}	126,24 ±2,728^{a}

**Different letters (a, b) in the same row represent statistically significant differences ($p < 0.05$) between treatments according to Tukey's test. Data are mean ± SE.*

3.6. Effects of SMG conditions on secondary compound accumulation in *Phyllanthus* callus and adventitious root cultured *in vitro*

The secondary compounds that determined the medicinal properties of Phyllanthus plant were rutin, and quercetin, which were flavonoids with a known role in preventing cancer cells, and hypophyllanthin and phyllanthin (lignans with the function of protecting the liver). HPLC analysis results showed that the contents of flavonoids (rutin and quercetin), and lignans (hypophyllanthin and phyllanthin) were different in SMG and G conditions (Table 3.6).

Table 3.6. The secondary compounds in *Phyllanthus* callus and adventitious root cultured *in vitro* after 4 weeks under SMG conditions

Treatment		**Flavonoid (µg/1 g fresh weight)**		**Lignan (µg/1 g fresh weight)**	
		Rutin	**Quercetin**	**Hypophyllanthin**	**Phyllanthin**
G	Callus	-**	9.61 ± 0.024^{a}	17.29 ± 0.008^{b}	11.53 ± 0.005^{b}
SMG	Callus	1.79 ± 0.047^{a*}	3.25 ± 0.007^{b}	29.06 ± 0.039^{a}	16.01 ± 0.041^{a}
	Adventitious root	1.19 ± 0.007^{b}	9.54 ± 0.066^{a}	9.03 ± 0.043^{c}	-

**Different letters (a, b) in the same row represent statistically significant differences ($p < 0.05$) between treatments according to Tukey's test. Data are mean ± SE.*

3.7. Effects of SMG conditions on energy metabolism (starch and sugar) in *Begonia* SEs cultured *in vitro*

During the first month, corresponding to the SEs induction, changes in starch and sugar levels under G and SMG conditions were recorded and shown in Fig. 3.9.

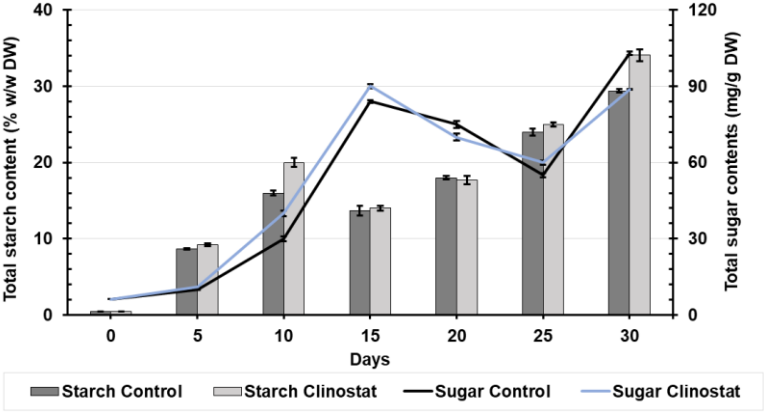

Figure 3.9. Effects of SMG conditions on starch and sugar contents in *Begonia* SEs cultured *in vitro*

The results also showed that the total starch content of the SMG treatment reached its maximum on the last day of culture of the 2nd month (Table 3.7) when the spherical SEs was differentiating into heart-shaped and torpedo-shaped. Then, the total starch content of the SMG treatment decreased after 2 and 3 months of culture coinciding with the maturation of SEs.

Table 3.7. Effects of SMG conditions on starch and sugar contents in Begonia SEs cultured *in vitro* after 1, 2, 3 and 4 months

Duration	**1 month**		**2 months**		**3 months**		**4 months**	
Content	**G**	**SMG**	**G**	**SMG**	**G**	**SMG**	**G**	**SMG**
Starch (% w/w DW)	29.37 ± 0.14^{g*}	34.06 ± 0.44^{e}	38.28 ± 0.09^{b}	39.91 ± 0.03^{a}	38.13 ± 0.06^{b}	35.06 ± 0.04^{d}	30.02 ± 0.03^{f}	35.91 ±0.10^{c}
Sugar (mg/g DW)	103.01 ± 0.07^{e}	88.74 ± 0.34^{f}	84.67 ± 0.23^{g}	68.19 ± 0.21^{h}	124.43 ± 0.65^{c}	153.61 ± 0.30^{a}	105.67 ± 0.31^{d}	150.00 ± 0.36^{b}

**Different letters (a, b) in the same row represent statistically significant differences ($p \leq 0.05$) between treatments according to Tukey's test. Data are mean ± SE.*

3.8. The growth of *Begonia* and Strawberry shoot or plantlet derived from SMG conditions under gravity

3.8.1. The growth of Begonia shoot or plantlet derived from SMG conditions under gravity

After 1 month of culture under G conditions, the explants derived from SMG showed the number of shoots (136.67 shoots), fresh weight (4774.67 mg), and dry weight (423.33 mg) reached the highest; However, shoot height (1.13 cm), leaf area (0.90 cm^2) and chlorophyll content (38.37 $nmol/cm^2$) reached significantly lower values compared to the G condition (Table 3.8).

Table 3.8. The growth of *Begonia* shoot derived from SMG conditions under gravity after 1 month of culture

Treatment	No. of shoots	Shoot height (cm)	No. of leaves	Leaf area (cm^2)	Chlorophyll ($nmol/cm^2$)	Fresh weight (mg)	Dry weight (mg)
G	67.00± 1.15*	2.00± 0.06	3.33± 0.33	2.03± 0.09	45.40 ± 0.12	4007.67± 3.84	327.67± 0.88
SMG	136.67 ± 1.20	1.13± 0.12	2.33± 0.33	0.90± 0.56	38.37 ± 0.16	4774.67± 13.84	423.33± 0.88
Sig. t-Test	0	0,003	0,101	0	0	0	0
	≠	≠	×	≠	≠	≠	≠

** Data are mean ± SE according to t-Test ($p < 0.05$).*
≠ Significant difference; × No difference.

The results showed that *Begonia* plants derived from SMG treatment obtained plantlet height (12.33 cm), number of main roots (8.67 cm), main root length (5.33 cm), number of leaves (6.67), chlorophyll (47.77 $nmol/cm^2$) and fresh weight (2212.00 mg), dry weight (217.33 mg) were significantly higher when compared to the control condition (Table 3.9).

Bảng 3.9. The growth of *Begonia* plantlet derived from SMG conditions under gravity after 3 months of culture

Nghiệm thức	Chiều cao cây (cm)	Số rễ chính	Chiều dài rễ chính (cm)	Số lá	Hàm lượng chlorophyll tổng (nmol/cm^2)	Khối lượng tươi (mg)	Khối lượng khô (mg)
G	9.00± 0.58*	6.67± 0.33	4.33 ± 0.33	4.67± 0.33	45.43± 0.09	2013.00± 1.15	203.33± 1.45
SMG	12.33± 0.33	8.67± 0.33	5.33 ± 0.33	6.67± 0.33	47.77± 0.09	2212.00± 0.58	217.33± 0.88
Sig. t-Test	0,007	0,013	0,101	0,013	0	0	0,01
	≠	≠	×	≠	≠	≠	≠

** Data are mean ± SE according to t-Test (p < 0.05).*
≠ Significant difference; × no difference.

3.8.2. The growth of Strawberry shoot or plantlet derived from SMG conditions under gravity

After 4 weeks of culture, shoot clusters under SMG conditions obtained the shoot height (2.90 cm), fresh weight and dry weight of shoot clusters (464 mg and 35.67 mg) was higher than those in G (Table 3.10). During the *in vitro* rooting stage, plant growth was similar to the shoot multiplication stage (Table 3.11).

Table 3.10. The growth of Strawberry shoot derived from SMG conditions under gravity after 4 weeks of culture

Treatment	No. of shoots	Dhoot height (cm)	Fresh weight (mg)	Dry weight (mg)	CAT (U/g)	APX (U/g)	Phenolic (mg GAE/100 g DW)
G	8.33± 0.33*	0.67 ± 0.03	351.33 ±5.78	26.00 ±0.58	167.85 ±0.26	0.13± 0.00	195.18 ±0.26
SMG	7.33 ±0.33	2.90 ± 0.12	464.00 ±6.66	35.67±0. 67	174.53 ±0.05	0.46± 0.02	153.36 ±0.26
Sig. t-Test	0,101	0	0	0	0	0	0
	×	≠	≠	≠	≠	≠	≠

** Data are mean ± SE according to t-Test (p < 0.05).*
≠ Significant difference; × no difference.

Table 3.11. The growth of Strawberry plantlet derived from SMG conditions under gravity after 2 weeks of culture

Treatment	Plantlet height (cm)	No. of leaves	Leaf width (cm)	No. of roots	Root length (cm)	Fresh weight (mg)	Dry weight (mg)
G	6.23 ± 0.15*	6.00 ± 0.00	1.10 ± 0.10	7.33 ± 0.67	4.67 ± 0.33	233.33 ± 17.64	26.00 ± 2.08
SMG	8.33 ± 0.33	6.67 ± 0.33	1.70 ± 0.10	9.33 ± 0.67	4.33 ± 0.33	653.33 ± 68.88	65.67± 3.38
Sig. t-Test	0,004	0,184	0,013	0,101	0,59	0,004	0,001
	≠	×	≠	×	×	≠	≠

** Data are mean ± SE according to t-Test (p < 0.05).*
≠ Significant difference; × no difference.

3.9. The proliferation of *Phyllanthus* callus derived from SMG conditions under gravity

After 4 weeks of culture, the results showed that two processes had taken place: proliferation and regeneration (shoots and roots) (Fig. 3.10C-D) under both SMG and G conditions. In addition, the callus fresh weight under SMG conditions was 1.67 times higher than under G conditions. Meanwhile, the callus dry weight under SMG conditions was also approximately 1.6 times higher than under G conditions (Table 3.12, Fig. 3.10A-B).

Table 3.12. The proliferation of *Phyllanthus* callus derived from SMG conditions under gravity after 4 weeks of culture

Treatment	Fresh weight (mg)	Dry weight (mg)	Flavonoid (µg/1 g FW)		Lignan (µg/1 g FW)		Hormone	
			Rutin	Quercetin	Hypophyllanthin	Phyllanthin	SA	MEL
G	1933.33 ±20.28*	196.67± 8.82	-**	10.49 ±0.01	18.70 ±0.04	12.40 ±0.30	1.46 ±0.002	0.2 ±0.0008
SMG	3213.33 ±34.80	310.00 ±11.55	2.39 ±0.02	17.67 ±0.02	39.77 ±0.02	29.03 ±0.04	1.59 ±0.006	0.39 ±0.003
Sig. t-Test	0	0,01	0	0	0	0	0	0
	≠	≠	≠	≠	≠	≠	≠	≠

** Data are mean ± SE according to t-Test (p < 0.05).*
*** No data recorded.*
≠ Significant difference; × no difference.

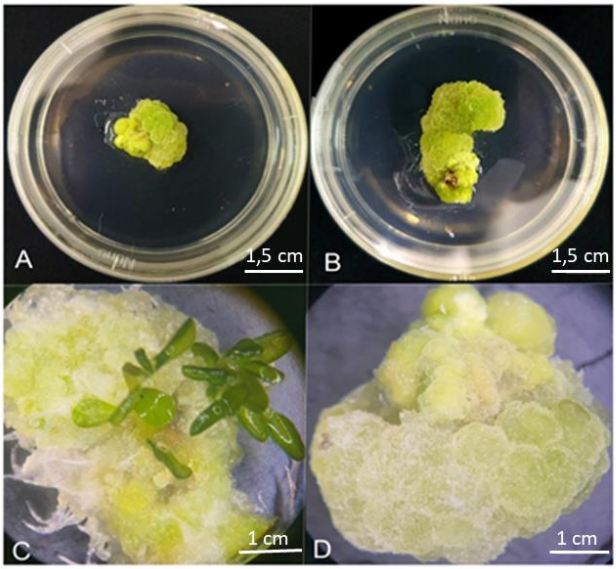

Figure 3.10. The proliferation of *Phyllanthus* callus derived from SMG conditions under gravity after 4 weeks of culture. **A:** Callus derived from under G; **B:** The proliferation of *Phyllanthus* callus derived from SMG conditions under gravity after 4 weeks of culture SMG; **C:** Shoot and adventitious root regenerated from callus**; D:** Calli observed under a stereo microscope with ×10 eyepiece.

3.10. The acclimatization of Strawberry plantlet derived from SMG conditions in the greenhouse

SMG derived- plantlets also recorded better acclimatization and growth as compared to G derived- plantlets after 4 weeks of planting (Table 3.13). In addition, the runner formation also recorded differences in the two culture conditions (Table 3.14 and Fig. 3.11).

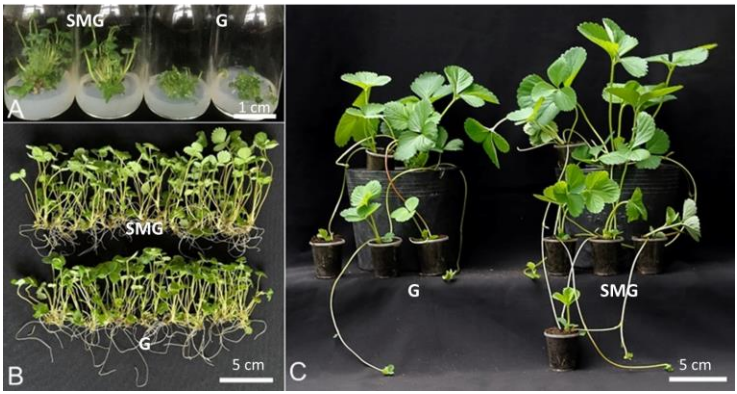

Figure 3.11. The growth of Strawberry shoot, plantlet, runner formation derived from SMG treatment in the greenhouse. **A:** Shoot multiplication after 4 weeks; **B:** 4-week-old plantlet in the greenhouse; **C:** Runner formation after 8 weeks of planting in the greenhouse.

Table 3.13. The acclimatization of Strawberry plantlet derived from SMG treatment in the greenhouse

Traetment	Plantlet height (cm)	No. of leaves	Leaf width (cm)	No. of roots	Root length (cm)	Fresh weight (mg)	Dry weight (mg)	Chlorophyll (nmol/cm^2)
G	11.37 ± 0.19*	8.00 ± 0.58	4.67 ± 0.09	10.33 ±0.88	11.20 ± 0.21	3775.67 ±102.28	431.00 ± 17.21	45.33 ± 1.45
SMG	12.63 ± 0.09	9.00 ± 0.58	5.17 ± 0.20	12.67 ±0.88	13.67 ± 0.90	4096.00 ±54.53	469.00 ± 10.58	48.00 ± 1.53
Sig. t-Test	0,004	0,288	0,087	0,135	0,056	0,051	0,133	0,275
	≠	×	×	×	×	×	×	×

** Data are mean ± SE according to t-Test ($p < 0.05$).*
≠ Significant difference; × no difference.

Table 3.14. The runner formation of Strawberry plantlet derived from SMG treatment in the greenhouse

Treatment	Runner formation time (day)	Runner formation (%)	No. of runner/ plantlet	Runner			
				Runner height (cm)	No. of leaves	Fresh weight (mg)	Chlorophyll (nmol/cm^2)
G	30.00 ± 0.58*	83.33 ± 3.33	4.00 ± 0.00	7.73 ± 0.63	4.00 ± 0.00	2049.67 ± 42.98	42.67 ± 0.80
SMG	27.67 ± 0.33	93.33 ± 3.33	5.33 ± 0.33	9.07 ± 0.15	4.33 ± 0.33	2336.33 ± 66.47	43.60± 0.68
Sig. t-Test	0,025	0,101	0,057	0,109	0,423	0,022	0,425
	≠	×	×	×	×	≠	×

** Data are mean ± SE according to t-Test ($p < 0.05$).*
≠ Significant difference; × no difference.

3.11. Proposing a process for micropropagation of *Begonia* and Strawberry plants and producing secondary compounds on an industrial scale on *Phyllanthus* plant

Based on the results of this study, the process of propagating *Begonia* and Strawberry plants and producing large-scale secondary compounds on *Phyllanthus* by SMG treatment during the in vitro morphogenesis was proposed attitude.

Chapter 4: CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

The results have shown the positive effect of SMG conditions on the *in vitro* morphogenesis and growth of *Begonia*, Strawberry and *Phyllanthus* plants.

1. *In vitro* morphogenesis

For *Begonia*, SMG conditions promoted the induction, differentiation and maturation of SEs from petiole explants cultured *in vitro*.

For Strawberry, SMG conditions simulated callus induction and shoot regeneration of *in vitro* leaf explants.

For *Phyllanthus*, SMG conditions promoted callus induction and adventitious root formation of *in vitro* internode explants.

2. The morphogenesis under gravity stress conditions has been adapted by adjusting the content and ratio of endogenous hormones (AUX, GA3, ABA and CKs) in *Begonia*, Strawberry and *Phyllanthus*. Or by enhancing the activity of antioxidant enzymes (CAT, APX) in Strawberry plants and *Phyllanthus* plants. This adaptation is also demonstrated by increased secondary compound accumulation and cell proliferation in *Phyllanthus*.

3. The shoot growth and *in vitro* rooting of *Begonia* plants in gravity from SMG derived- SEs were also enhanced; SMG derived-

plantlets grew and developed well and had no morphological abnormalities.

For Strawberry, the growth of shoot and planlet derived from SEs under SMG were higher than those in G conditionsthe subsequent growth and runner formation of SMG derved- plantlets were not significant as compared to those in G derived- plantlets (excepted runner time of SMG derived- planelets was earlier)

4. For *Phyllanthus*, callus proliferation formed under SMG condition for biomass and secondary compound accumulation of flavonoids (rutin and quercetin) and lignans (phyllanthin and hypophyllanthin) was shown to be better than under G condition.

4.2. Recommendations

1. Continuing record the growth of *Phyllanthus* shoot and root derived from SMG treatment.

2. Continuing record the acclimatization of *Begonia* and *Phyllanthus* plantlets derived from SMG treatment in the greenhouse.

3. Researching SMG conditions on other plants with different simulation devices and rotation speeds; especially delve into the role of SMG in biochemical mechanisms and gene expression.

4. Researching and applying explants under SMG conditions in the field of micropropagation and cell culture to produce secondary compounds on an industrial scale.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Hoang Dac Khai, **Le The Bien**, Nguyen Quang Vinh, Doan Manh Dung, Ngo Dai Nghiep, Nguyen Thi Nhu Mai, Hoang Thanh Tung, Vu Quoc Luan, Do Manh Cuong, Duong Tan Nhut, *Alterations in endogenous hormone levels and energy metabolism promoted the induction, differentiation and maturation of Begonia somatic embryos under clinorotation*, Plant Science, 2021, 312, 111045.

2. **Le The Bien**, Hoang Thanh Tung, Nguyen Thi Nhu Mai., Truong Hoai Phong, Do Manh Cuong, Hoang Dac Khai, Vu Quoc Luan, Nguyen Ba Nam, Trinh Thi Huy Tra, Bui Van The Vinh, Duong Tan Nhut, *Morphogenesis of in vitro strawberry leaf cultured under Clinostat 2-D condition*, Plant Cell, Tissue and Organ Culture, 2023, 152(1), 1-12.

3. **The Bien Le,** Thanh Tung Hoang, Thi Nhu Mai Nguyen, Dac Khai Hoang, Manh Cuong Do, Hoai Phong Truong, Quoc Luan Vu, Van The Vinh Bui, Tan Nhut Duong, *In vitro morphogenesis, antioxidant enzyme activity and secondary compound accumulation of Phyllanthus amarus under Clinostat 2D condition*, Vietnam Journal of Science and Technology B, 2024, 66(2), 49-54.

4. Duong Tan Nhut, Hoang Dac Khai, Nguyen Xuan Tuan, **Le The Bien**, Hoang Thanh Tung, *In vitro growth and development of plant under simulated microgravity condition*, In: Duong Tan Nhut, Hoang Thanh Tung, Edward Chee-Tak Yeung (Eds), *Plant tissue culture: New techniques and application in horticultural species of tropical region*, Springer, Singapore, 2022, 343-382.