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THE CORRELATION BETWEEN HORMONES AND MOPHOGENESIS THROGH THIN CELL LAYER CULTURE TECHNIQUE IN *Passiflora* L., *Gerbera* L., AND *Phyllanthus amarus*

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INTRODUCTION

The emergence of morphological forms is a complex biological process that plays a crucial role in the development of plants and is particularly significant in the field of *in vitro* tissue culture. In the context of the expanding application of biotechnology in agriculture, medicinal plants, and ornamental flowers, mastering the mechanism of morphological development will help enhance the efficiency of propagation, breed new varieties, and produce large-scale crops under controlled conditions. One of the key factors controlling this process is endogenous hormones - growth regulators that regulate cell division, elongation, and differentiation in plants. However, the complex interactions between these hormones and their effects on different stages of organogenesis remain unclear, especially in high-value crops.

The Thin Cell Layer (TCL) culture technique was developed to improve regeneration efficiency and minimize variability in tissue culture. The advantage of TCL is that it uses a limited number of cells in the culture, providing high uniformity, thus allowing for a clearer analysis of the effects of physiological factors such as endogenous hormones. This method has been applied to some plant species, such as orchids, medicinal plants, and vegetables, but has not been fully explored in other crop groups, such as fruit trees, cut flowers, or medicinal plants.

Plant hormones (phytohormones) like auxin, cytokinin, gibberellin, ethylene, and abscisic acid play an important role from embryogenesis to plant senescence. These hormones do not act independently but often interact with each other, either stimulating or inhibiting growth. In addition to endogenous hormones, plant growth regulators (PGRs), which are synthetic chemical compounds, are also widely used in tissue culture to promote processes such as callus formation, embryo development, shoot regeneration, root formation, and flowering. However, the effectiveness of PGRs depends on their positive

interaction with endogenous hormones, requiring clarification of the complex relationship between them to ensure healthy plant growth.

Based on this practical background, the thesis titled 'Study on the correlation between hormones and the ability to induce morphology through Thin Cell Layer culture technique on passion fruit, gerbera, and *P. amarus*' was carried out with the aim of analyzing the relationship between fluctuations in endogenous hormone levels and the potential for *in vitro* morphological development in three representative plant species.

Research objectives: To determine the correlation between endogenous hormone levels and the ability to induce morphological development through the TCL culture technique in passion fruit, gerbera, and *Phylanthus amarus*.

Research subjects: he correlation between endogenous hormones (IAA, CKs, GA3, ABA, SA, JA, MEL, and ET) and the ability to induce morphological development through the TCL culture system in passion fruit, gerbera, and *Phylanthus amarus*.

New contributions of the thesis: This study is the first to apply the TCL culture technique to systematically evaluate the correlation between endogenous hormones, ethylene, and antioxidant system activity with the process of morphological development (adventitious shoots, somatic embryos, callus, and adventitious roots) in three plant groups with different biological and economic values, including fruit crops (passion fruit), cut flower crops (gerbera), and medicinal plants (*Phyllanthus amarus*). In this research, the thesis provides some of the latest scientific data.

Structure of the thesis: The thesis includes 5 main sections: Introduction, Chapter 1: Overview (24 pages), Chapter 2: Content, materials, and Methods (15 pages); Chapter 3: Results and Discussion (62 pages), and Conclusion and Recommendations (2 pages) section.

Chapter 1. OVERVIEW

1.1. Plant morphogenesis

Morphogenesis is a fundamental biological process that reflects the formation and differentiation of plant structures throughout development, through the organization and reorganization of cells, tissues, and organs under the control of genetic potential and intrinsic physiological signals.

Under *in vitro* culture conditions, the regeneration of whole plants mainly occurs *via* two pathways: organogenesis and somatic embryogenesis. Both are based on the totipotency of somatic cells and are regulated by endogenous hormones, particularly the balance between auxin and cytokinin.

Regeneration may occur directly or indirectly through callus tissue, which is a mass of proliferating, unorganized cells that retains the potential to regenerate organs or somatic embryos depending on physiological status and culture conditions.

In vitro morphogenesis is influenced by genotype, explant source, culture conditions, and wound signals; therefore, *in vitro* culture serves as an effective tool for studying the mechanisms of plant morphogenesis under controlled conditions.

1.2. Thin cell layer Culture Technique

The thin cell layer (TCL) culture technique is an effective tool for studying the mechanisms of *in vitro* morphogenesis because it uses explants of small size, simple tissue structure, and high uniformity. These characteristics help minimize physiological interference among different tissue types, thereby highlighting the regulatory role of endogenous hormones in specific morphogenetic pathways such as adventitious shoot regeneration, somatic embryogenesis, callus formation, and adventitious root development. Through the TCL technique, the relationship between fluctuations in endogenous hormone levels and morphogenetic potential can be analyzed more clearly,

contributing to a better understanding of hormone regulatory mechanisms specific to each plant species and each morphogenetic process.

1.3. Plant endogenous hormones

1.3.1. Definition

1.3.2. Activity of endogenous hormones

1.3.3. Interaction of endogenous hormones in plant

Endogenous plant hormones are chemical substances that act at very low concentrations and play regulatory roles in cell and tissue growth, development, and differentiation. Unlike animals, plants do not possess specialized glands for hormone production and storage; therefore, hormones are synthesized in a dispersed manner, utilized locally, or passively transported within the plant through cytoplasmic streaming, intercellular diffusion, and the vascular tissue system.

Not all cells respond to hormones; only those cells that are programmed to respond at specific developmental stages and physiological locations are sensitive to hormonal signals. Plants possess intrinsic mechanisms to regulate hormone levels and activity by controlling hormone biosynthesis, transport, storage, inactivation, or degradation when they are no longer required. Because endogenous hormones occur at extremely low concentrations (10^{-6}–10^{-5} mol/L), their study is technically challenging and only became well developed from the late 1970s onward, mainly through genetic studies and *in vitro* culture research.

1.4. Overview of research subjects

1.4.1. Passion fruit

1.4.2. Gerbera

1.4.3. Phyllanthus amarus

Chapter 2. CONTENTS, MATERIALS, AND METHODS

2.1. Research content

Content 1: Study on selecting the optimal explant source for *in vitro* morphological development in passion fruit, gerbera, and *Phyllanthus amarus*.

Content 2: Study on the correlation between endogenous hormone levels and *in vitro* morphological development in passion fruit, gerbera, and *P. amarus*.

Content 3: Study on the impact of the thin cell layer (TCL) culture technique on ethylene accumulation and the antioxidant system during the development of shoots in passion fruit, somatic embryos in gerbera, and advetitiou roots in *P. amarus.*

2.2. Research materials

2.2.1. Plant materials

The study uses *in vitro* stem cuttings of passion fruit, ex vitro florescent of gerbera, and *in vitro* stem cuttings of *P. amarus* from the Molecular Biology and Crop Breeding Laboratory at the Taynguyen Institute for Scientific Research (now the Institute of Life Science) as initial materials.

For the study analyzing the correlation between hormone levels and morphological development, the following materials were used: regenerated shoots from stem cuttings and TCL stem cuttings of passion fruit, somatic embryos from flower bases of gerbera, and callus, regenerated roots from stem cuttings of *P. amarus*.

2.2.2. Equipment, instruments, and chemicals

2.2.3. Culture media

2.3. Research methods

2.3.1. Experimental design

2.3.1.1. Content 1: Study on selecting the optimal explant source for in vitro morphological development in passion fruit, gerbera, and P. amarus

- Experiment 1: Study on the influence of explant position and endogenous CKs and AUX content in the initial explant on the shoot

regeneration ability of *in vitro* stem cuttings of passion fruit: 10 mm stem segments from 2-month-old *in vitro* passion fruit shoots taken from internodes 1 to 4 (counting from the shoot tip). These explants were cultured on a shoot regeneration medium to compare shoot regeneration abilities from different stem node positions. The endogenous hormone content (CKs and IAA) in the initial explant and the percentage of morphological development (%) after 60 days of culture were recorded in this study.

- Experiment 2: Study on the influence of flower base age and endogenous CKs and AUX content in the initial explant on the somatic embryo regeneration ability of gerbera cultured *in vitro*: The ex vitro flower bases of gerbera at three different developmental stages (flower bud, open calyx, and colored flower rays) were sterilized. The study also recorded the endogenous hormone content (CKs and IAA) in the initial explants and the percentage of morphological development (%) after 90 days of culture.
- Experiment 3: Study on the influence of explant position and endogenous CKs and AUX content in the initial explant on the callus regeneration ability of *in vitro* stem cuttings of *P. amarus*: Stem segments (10 mm) of 2-month-old *in vitro* shoots of *P. amarus*, taken from stem nodes 1 to 3 (counting from the shoot tip), were cultured on a callus induction medium to compare the callus formation ability at different stem node positions. The endogenous hormone content (CKs and IAA) in the initial explants and the percentage of morphological development (%) after 40 days of culture were recorded in this study.
- Experiment 4: Study on the impact of the Thin cell layer (TCL) culture technique on shoot regeneration ability of passion fruit, gerbera, and P. amarus cultured *in vitro*:

The stem segment of 2-month-old *P. amarus* (third stem node counting from the top) was cut into two equal lTCL with dimensions of 0.5 × 10 mm and

cultured on a shoot regeneration medium. The morphological development process was recorded after 10, 30, 60, and 90 days of culture.

The flower base explants of gerbera were cut into 4 equal TCL with dimensions of 0.5 × 10 mm and cultured on a somatic embryo induction medium. The control group was the intact flower base. The morphological development process was recorded after 30, 60, 90, and 120 days of culture.

The stem segments of 2-month-old *P. amarus* (the second stem node for callus formation and the third stem node for adventitious root formation) were cut into two equal lTCL with dimensions of 0.5 × 10 mm and cultured on a shoot/adventitious root regeneration medium (Fig. 6). The morphological development process was recorded after 10, 20, 40, and 60 days of culture.

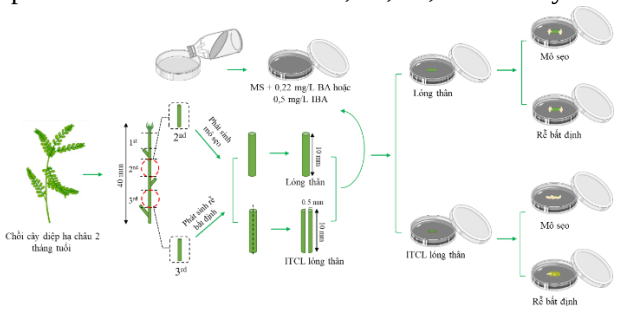

Figure 2.6. Experimental procedure for in vitro morphogenesis of Phyllanthus internode explants using the thin cell layer (TCL) culture technique.

2.3.1.2. Content 2: Study on the correlation between endogenous hormone levels in the morphological development of passion fruit, gerbera, and P. amarus

- Experiment 1: Study on the fluctuations of endogenous hormone levels during the *in vitro* morphological development of passion fruit, *P. amarus*, and gerbera: Fresh samples (adventitious shoots of passion fruit, somatic embryos of gerbera, callus and adventitious roots of *P. amarus*) collected from previous experiments were used as source materials for endogenous hormone analysis.
- Experiment 2: Study on the correlation between endogenous hormone levels in the morphological development process of passion fruit, gerbera, and

P. amarus: The hormone content in the explants (shoots of passion fruit, somatic embryos of gerbera, and callus and adventitious roots of *P. amarus* cultured *in vitro*, as analyzed in the previous experiment) was analyzed for correlation and presented as a heatmap diagram (Pearson correlation) using OriginPro 2024b software.

2.3.1.3. Content 3: Study on the impact of the thin cell layer (tcl) culture technique on ethylene accumulation, antioxidant system activity, and secondary metabolite synthesis during the morphological development of passion fruit, gerbera, and *P. amarus*

- Experiment 1: Study on the impact of the TCL culture technique on ethylene accumulation during the morphological development of passion fruit shoots, gerbera somatic embryos, and *P. amarus* adventitious roots: To determine the accumulated ET concentration in the culture system, gas chromatography with a flame ionization detector (GC/FID) was used in this study.

- Experiment 2: Study on the impact of the TCL culture technique on the antioxidant system during the morphological development of passion fruit shoots, gerbera somatic embryos, and *P. amarus* adventitious roots: Fresh samples (0.3g) were ground with liquid nitrogen and extracted in 0.1M phosphate buffer (pH 7.4) containing EDTA. The supernatant after centrifugation was used to determine the activity of antioxidant enzymes such as SOD, CAT, and APX, along with DPPH free radical scavenging ability, total phenolic content, and vitamin C. SOD activity was determined by its ability to inhibit pyrogallol oxidation at a wavelength of 320 nm; CAT activity was measured by its reaction with H_2O_2 to form a yellow complex with ammonium molybdate at 405 nm; APX was measured by the Nakano and Asada method at 290 nm. The DPPH free radical scavenging ability was calculated as the RSA percentage. Total phenolic content was determined using Folin-Ciocalteu

reagent and expressed as mg GAE/100g dry weight. Vitamin C was quantified by titration with 2,6-dichlorophenol-indophenol dye.

- Experiment 3: Study on the impact of the TCL culture technique on secondary metabolite synthesis in *P. amarus* adventitious roots: Ethanol 80% extracts of adventitious roots from stem segments and TCL stem segments were analyzed using the Thermo-Ultimate 3000 HPLC system (Thermo Scientific, USA). The calibration curves and chromatograms were used to quantify these compounds (Fig. 2.10).

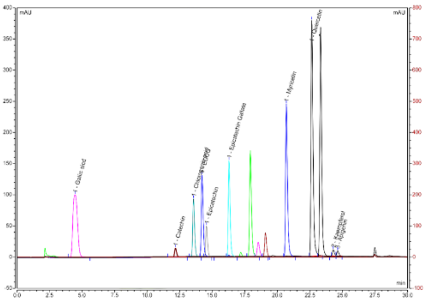

Figure 2.10. Chromatograms of the nine secondary compounds analyzed.

2.3.2. Some methods and techniques used in Figure 2.10. Chromatograms of the nine secondary compounds analyzed.the study

2.3.2.1. Sample collection and evaluation of some indicators in the study

2.3.2.2. Morphological observation

Cellular changes during morphological development were monitored using histological methods. Observations were carried out under an optical microscope with 10× eyepiece and 10× and 40× objective lenses.

2.3.2.2. Data analysis and processing

All collected data corresponding to each monitoring indicator were entered into Microsoft Excel 2016. The data were processed and categorized using SPSS 20.0 software with Duncan's test at a significance level of $p < 0.05$. Graphs (± SD) were plotted using Microsoft Excel 2016, and heatmaps were processed using OriginPro 2024b (Pearson correlation).

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Content 1: study on selecting the optimal explant source for *in vitro* morphological development in passion fruit, gerbera, and *P. amarus*

3.1.1. Influence of explant position and endogenous cks and aux content in the initial explant on shoot regeneration ability of in vitro stem segments of passion fruit

The study indicates that the position of the explant on the stem of passion fruit significantly affects the shoot regeneration ability. At internode 1, the regeneration rate was only 40%, with small shoots. This rate increased to 63% at internode 2 and reached 93.33% at internode 3, indicating that this is the optimal position. In contrast, stem node 4 showed no shoot regeneration.

UHPLC-UV analysis revealed that the IAA concentration gradually increased from stem node 1 to node 4 (8.958 µg/g FW to 23.848 µg/g FW), while the CKs concentration decreased from internode 1 to 4 (6.548 µg/g FW to 3.891 µg/g FW). The IAA/CKs ratio increased significantly from 1.368 at stem node 1 to 6.129 at internode 4, showing an increasing influence of AUX compared to CKs at the more distal positions of the stem.

3.1.2. Influence of flower base age and endogenous cks and aux content in the initial explant on somatic embryo regeneration ability of gerbera in in vitro culture

The age of the flower base significantly affects the somatic embryo regeneration ability of gerbera *in vitro*. The flower bud stage showed the highest regeneration rate (100%) with 30.67 somatic embryos, due to the young cells and strong physiological activity. As the flower developed, the regeneration rate gradually decreased, reaching only 12% at the colored flower ray stage.

The highest CKs content was observed at the flower bud stage, which helped promote cell division and somatic embryo development. The CKs level gradually decreased in subsequent stages. The AUX/CKs ratio at the flower bud

stage was 1.923, indicating a balance between these two hormones, which provided favorable conditions for tissue regeneration and differentiation.

3.1.3. Influence of explant position and endogenous cks and aux content in the initial explant on callus regeneration ability of in vitro stem segments of P. amarus

The study showed differences in the callus and adventitious root regeneration ability of *P. amarus* at different stem node positions. Explants from internode 1 and 2 formed callus at 100% regeneration rate, but internode 3 not only formed callus but also induced adventitious roots, due to the high AUX concentration and cellular differentiation at this position. Stem node 3, being closer to the root system, showed stronger root regeneration ability. The CKs concentration decreased as the explant moved closer to the roots, while the AUX concentration increased. The AUX/CKs ratio at all three stem node positions was approximately 1 or higher, indicating callus formation ability. The lowest AUX/CKs ratio was found at stem node 1 (0.970), and the highest at stem node 3 (4.203), reflecting differences in morphological development ability. The high AUX/CKs ratio at stem node 3, despite the addition of exogenous CKs, still indicated the potential for adventitious root formation.

3.1.4. The impact of the thin cell layer (tcl) culture technique on morphological development in passion fruit, gerbera, and P. amarus

The results from the study on the TCL culture technique showed a clear difference in morphological development ability compared to stem segments in passion fruit, *P. amarus*, and gerbera.

For passion fruit, both stem segments and TCL stem segments were capable of regenerating adventitious shoots. However, the TCL stem segments showed superior growth, with the number of shoots increasing from 2.33 shoots on day 15 to 12.33 shoots on day 90, along with stronger shoot height growth (from 1.54 cm to 3.45 cm) (Fig. 3.11). The regeneration coefficient of TCL stem

segments was much higher, reaching 24.67 on day 90, far surpassing the stem segment control (Fig. 3.11).

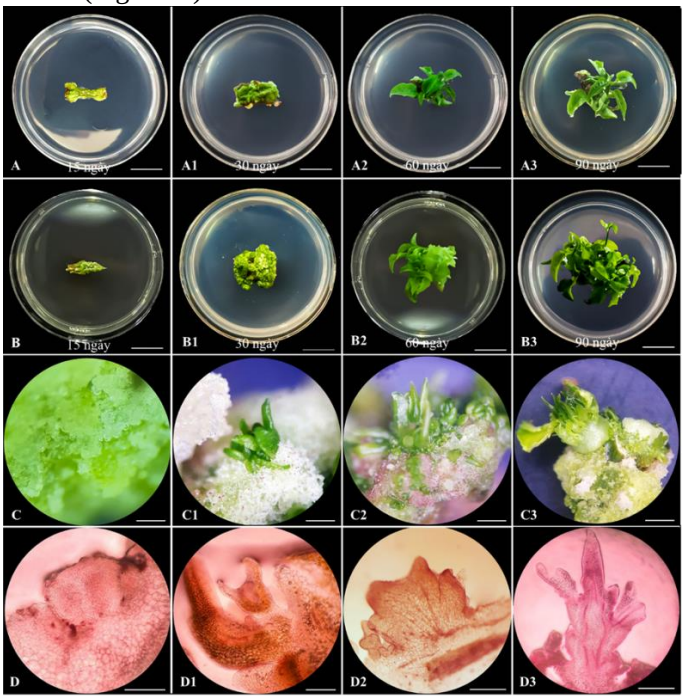

Figure 3.11. Indeterminate shoot formation from stem segment explants and TCL stem segment explants of passion fruit. A-A3. Indeterminate shoots derived from stem segment explants (Bar = 2 cm). B-B3. Indeterminate shoots derived from TCL stem segment explants (Bar = 2 cm). C-C3. Indeterminate shoot induction in B-B3 observed under a stereo microscope (Bar = 0.3 cm). D-D3. Indeterminate shoots in B-B3 under a light microscope at ×10 magnification (Bar = 40 µm).

For gerbera, the TCL florescent method enhances somatic embryo formation compared to the regular flower base explant. The number of somatic embryos increased significantly in the TCL flower base, rising from 19.33 embryos/explant on day 30 to 83.67 embryos/explant by day 120 (Fig. 3.13). The transition from spherical embryos to heart-shaped embryos and the

development of cotyledons also occurred more rapidly in the TCL samples, particularly by day 60 (Fig. 3.13).

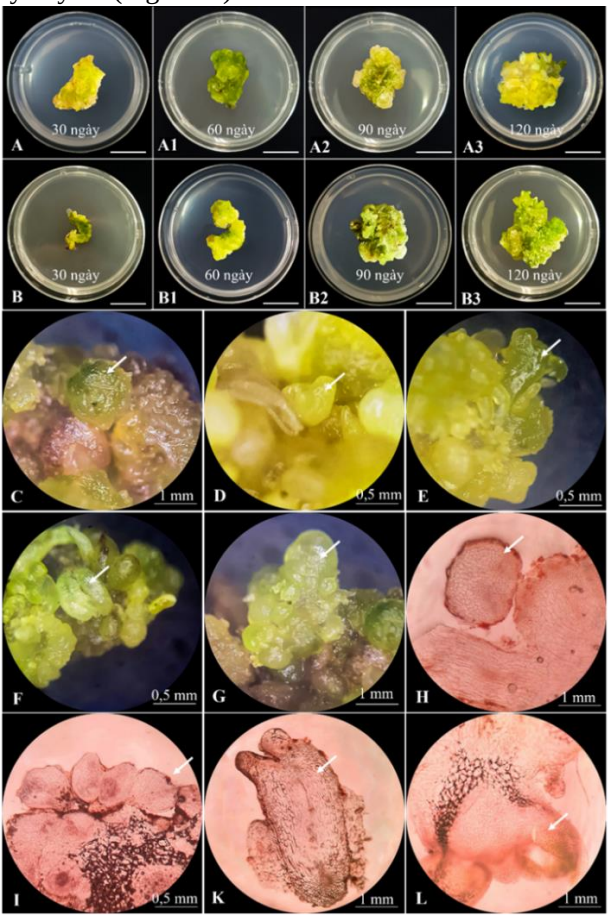

Figure 3.13. Somatic embryo formation from the receptacle explant and lTCL receptacle explant of gerbera. A–A3. Somatic embryos derived from the receptacle explant (Bar = 2 cm). B–B3. Somatic embryos derived from the lTCL receptacle explant (Bar = 2 cm). C. Somatic embryo at the globular stage. D. Somatic embryo at the heart-shaped stage. E. Somatic embryo at the torpedo stage. F. Somatic embryo at the cotyledonary stage. G. Polyembryonic somatic embryo. H. Histological section of a globular-stage somatic embryo. I. Histological section of a heart-shaped somatic embryo. K. Histological section of a torpedo-stage somatic embryo. L. Histological section of a two-cotyledon somatic embryo.

For *P. amarus*, both stem segments and TCL stem segments formed callus, but TCL stem segments gave superior results. The callus formed more quickly, covering the entire explant by day 10 (Fig. 3.15). Adventitious root formation was also more optimal in TCL samples, with longer roots and more fine roots after 60 days of culture. In contrast, stem segments had fewer roots, and the root tips were yellow.

In conclusion, the TCL technique significantly enhances morphological regeneration, callus development, and adventitious root formation, producing superior results compared to stem segments in all three plant species studied.

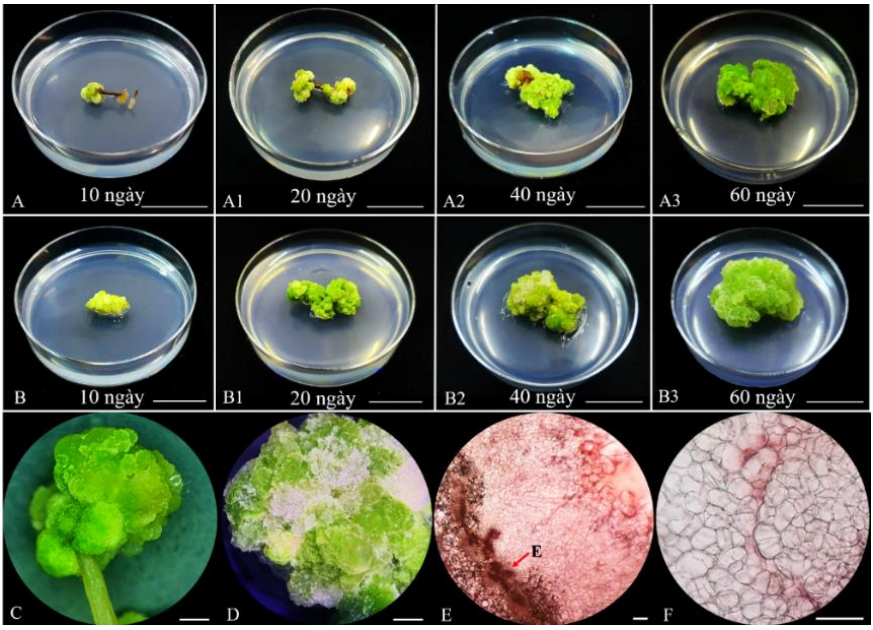

Figure 3.15. Callus formation from stem segment explants and TCL stem segment explants of Andrographis paniculata. A-A3. Callus derived from stem segment explants (Bar = 2 cm). B-B3. Callus derived from TCL stem segment explants (Bar = 2 cm). C. Callus in A1 observed under a stereo microscope (Bar = 0.3 cm). D. Callus in B1 observed under a stereo microscope (Bar = 0.3 cm). E. Callus in B3 under a light microscope at ×10 magnification (Arrow: Explant - E) F. Callus in B3 under a light microscope at ×40 magnification (Bar = 40 µm).

3.2. Content 2: The correlation of endogenous hormone levels during *in vitro* morphological development of passion fruit, gerbera, and *P. amarus*

3.2.1. Fluctuations and correlation of endogenous hormones during adventitious shoot regeneration in passion fruit

The results of the study indicate that plant hormones such as CKs, GA3, ABA, IAA, SA, and MEL show significant fluctuations during the tissue culture of passion fruit. Specifically, CKs dropped sharply on day 15, then increased significantly on day 30, before decreasing again on days 60 and 90. Other hormones, like GA3, ABA, and IAA, also showed a decreasing trend in the early stages, with clear fluctuations in the later stages.

Particularly, the ratios of hormones such as IAA/CKs, IAA/GA3, CKs/GA3, IAA/ABA, CKs/ABA, and GA3/ABA indicated the regulation and coordination between these hormones during the process of adventitious shoot formation, helping to stimulate cell division and development.

There were differences in hormone ratios between stem segment explants and TCL stem segment explants, with the TCL stem segments showing a strong antagonistic relationship between CKs and IAA, as well as a synergistic effect between GA3 and ET, which enhanced shoot regeneration ability. Moreover, the CKs concentration in TCL stem segments fluctuated strongly, indicating the strong influence of this hormone on shoot formation. Supporting hormones like SA and MEL also played a critical role in reducing stress and improving shoot emergence.

From these results, the study concluded that the TCL stem segment explants had higher shoot regeneration efficiency compared to stem segment explants, due to the optimal regulation of hormones such as CKs, GA3, IAA, and ABA, making the shoot formation process more efficient and adaptable.

3.2.2. Fluctuations and correlation of endogenous hormones during somatic embryo formation in gerbera

The study results reveal significant changes in endogenous hormone levels during the process of somatic embryo formation in gerbera across different culture stages. Throughout the culture process, most hormones, such as CKs, GA3, SA, ABA, JA, IAA, and MEL, showed strong fluctuations. These hormones typically decreased in the early stages (day 30) and gradually increased by day 60, then decreased again in the following days (90 and 120).

Specifically, CKs showed stable fluctuations on days 30 and 60, but then gradually decreased in the later stages for the flower base explant. In contrast, the TCL flower base explant exhibited a sharp increase on day 60 but decreased again on days 90 and 120. Hormones like GA3 and SA both increased on days 60 and 90, then decreased on day 120.

ABA levels varied between the two explant types: In the TCL flower base explant, ABA increased on day 60 and then decreased in subsequent days, while in the regular flower base explant, ABA continued to increase on day 90 and then sharply decreased on day 120. Similarly, JA levels increased on days 60 and 90 and decreased on day 120 for both explant types.

Ratios between hormones such as IAA/CKs, IAA/GA3, CKs/GA3, IAA/ABA, CKs/ABA, and GA3/ABA showed notable changes throughout the culture period. The IAA/CKs ratio increased on day 30 but decreased gradually until the end of the culture. The CKs/GA3 ratio increased on day 30, then decreased on day 60, and increased again on days 90 and 120 for the flower base explant, while this ratio in the TCL flower base explant showed an opposite trend. The GA3/ABA ratio in the TCL flower base explant increased sharply on day 30, then decreased sharply, and increased again on days 90 and 120.

The study suggests that the changes in hormone ratios like IAA, CKs, GA3, and ABA reflect the complex interactions between physiological and biochemical factors during somatic embryo formation. The correlation between

hormones in the flower base explant and TCL flower base explant also showed clear differences. In the flower base explant, the correlation between hormones was weak, while in the TCL flower base explant, hormones worked in synchrony and coordinated more closely, reflecting changes in the hormonal regulatory network depending on the type of explant used. This ultimately affects the efficiency and mechanism of somatic embryo development in each explant type.

3.2.3. Fluctuations and correlation of endogenous hormones during callus and adventitious root regeneration in P. amarus

This study analyzes the fluctuations of endogenous hormones during callus and adventitious root formation in *P. amarus* from two explant types: stem segments and TCL stem segments. The results show that, during callus formation, most hormones decreased sharply during the first 10 days of culture, except for CKs in the callus from stem segments, IAA in the callus and adventitious roots from stem segments, and ABA in the callus for both explant types. The CKs concentration gradually increased from day 1 to day 20 and decreased afterward, with a sharp increase on day 60 for stem segments. On the other hand, the TCL stem segments showed an increase in hormones on day 20, followed by a gradual decrease on days 40 and 60.

For adventitious root formation, the hormones decreased on day 10, then increased on day 20, except for SA. The TCL stem segments showed a sharp increase in CKs, IAA, and ABA on day 40, while stem segments exhibited inconsistent changes, with SA decreasing and MEL gradually increasing.

The fluctuations of hormone ratios such as IAA/CKs, IAA/GA3, IAA/ABA, CKs/GA3, CKs/ABA, and GA3/ABA indicated clear differentiation between the two explant types. The TCL stem segments exhibited stronger hormonal fluctuations, particularly the CKs/GA3 and IAA/CKs ratios. The correlation between hormones also showed distinct differences, with the stem segments showing a negative correlation between CKs and IAA, GA3, and

ABA, while in TCL stem segments, CKs had a positive correlation with all the other hormones.

The study results suggest that TCL stem segments have a better ability to induce callus and adventitious roots, with a stronger response to CKs and a shorter induction time, as well as better biomass accumulation. This explains why TCL stem segments are more effective in forming adventitious roots and callus, especially when combined with hormones like ABA and GA3.

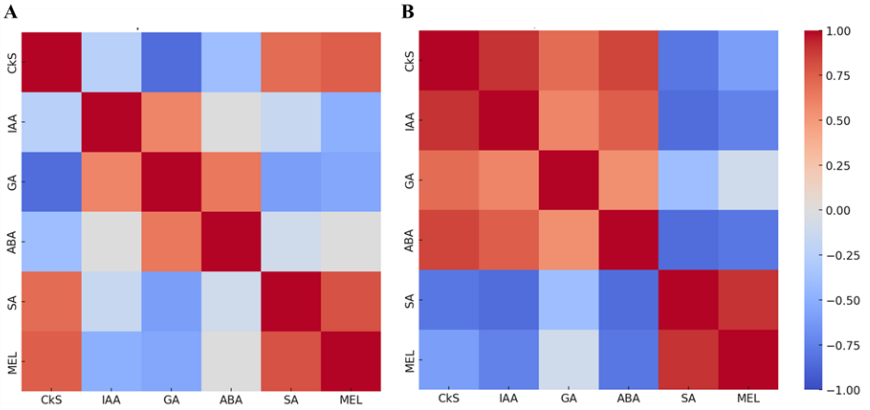

Figure 3.28. The correlation of endogenous hormones in callus derived from stem segments (A) and TCL stem segments (B) of *P. amarus*. The correlation coefficients were calculated based on Pearson correlation. Red indicates a positive correlation, while blue indicates a negative correlation.

3.2.2. Synthesis of secondary metabolites from callus and adventitious roots derived from tcl stem segments of P. amatus

The results of secondary metabolite synthesis (hypophyllanthin and phyllanthin) from callus and adventitious roots derived from TCL stem segments were recorded after 60 days of culture.

The study showed that callus produced 7.06 µg/g of hypophyllanthin, while adventitious roots produced a significantly higher amount, 11.54 µg/g. This indicates that adventitious roots exhibit a stronger biosynthesis of this compound. The presence of hypophyllanthin in both samples suggests the

production of biologically active lignans, which are commonly associated with various health benefits, such as antimicrobial and antioxidant properties.

In contrast, there was a stark difference in the synthesis of phyllanthin, with phyllanthin being undetectable in the callus, while adventitious roots synthesized 16.01 µg/g. This highlights that adventitious roots of *P. amarus* are the main source of phyllanthin, emphasizing their higher biosynthetic capacity for this particular lignan.

3.3. Content 3: The effect of the tcl culture technique on ethylene accumulation, antioxidant system activity, somatic embryo formation of passion fruit, and gerbera, *P. amarus*, and secondary metabolite synthesis in *P. amarus*

3.3.1. Effect of tcl culture technique on ethylene accumulation during shoot formation in passion fruit, somatic embryo formation in gerbera, and adventitious root formation in P. amarus

This study analyzes ethylene (ET) accumulation and its correlation with the process of shoot, somatic embryo, callus, and adventitious root formation in passion fruit, gerbera, and *P. amarus*.

In passion fruit, the TCL stem segments accumulated significantly more ET than the stem segments, with a sharp increase from 0.00 ppm to 6.15 ppm over 90 days, compared to 3.79 ppm in the stem segments. The correlation between ET and the number of adventitious shoots, as well as shoot height, was very strong, indicating that ET plays a vital role in stimulating cell division and shoot development.

For gerbera, the TCL flower base explants accumulated more ET than the regular flower base explants, with ET reaching 9.76 ppm on day 120, compared to 6.98 ppm in the regular flower base. The correlation between ET and the number of somatic embryos, particularly the cotyledon-stage embryos, was very

strong, suggesting that ET promotes somatic embryo formation and differentiation.

In *P. amarus*, the TCL stem segments accumulated higher ET than the stem segments, with ET concentrations of 9.83 ppm compared to 3.41 ppm after 60 days. The correlation between ET and the rate of adventitious root formation was 0.6125, indicating a moderate impact, while the relationship was stronger with fresh and dry biomass, suggesting that ET directly influences biomass accumulation and may indirectly promote adventitious root formation.

In summary, ET positively impacts the processes of shoot, somatic embryo, and adventitious root formation, with stronger effects in the TCL samples compared to the stem segment controls, providing insights into the potential application of adjusting ET concentrations to optimize tissue culture processes.

3.2. Effect of tcl culture technique on antioxidant enzyme activity during shoot formation in passion fruit, somatic embryo formation in gerbera, and adventitious root formation in P. amarus

This study evaluates the activity of enzymes and antioxidant compounds in the stem segments and TCL stem segments of passion fruit, gerbera, and *P. amarus.*

In passion fruit, the TCL stem segments exhibited stronger antioxidant activity than the stem segments, with higher activity levels in enzymes such as SOD, CAT, APX, and a greater ability to scavenge DPPH free radicals. The TCL stem segments also contained higher levels of phenolic compounds, contributing to superior antioxidant capacity.

For gerbera, the TCL flower base explants had higher antioxidant enzyme activity than the regular flower base explants, including SOD, CAT, APX, DPPH, and phenolic content. The TCL flower base explants showed a stronger ability to neutralize free radicals and protect cells, which helped enhance somatic embryo formation.

In *P. amarus*, the TCL stem segments showed higher antioxidant activity than the stem segments in all enzymes like SOD, CAT, APX, and DPPH. The TCL stem segments also had higher levels of non-enzyme antioxidants, such as phenolic compounds and vitamin C, demonstrating a stronger protective mechanism during adventitious root formation.

In conclusion, the TCL stem segments from all three species exhibited stronger antioxidant capacities, contributing to the development and protection of tissue culture samples.

3.3. Synthesis of secondary metabolites in adventitious roots derived from stem segments and tcl stem segments of P. amarus

The levels of secondary metabolites between the two explant types—stem segments and TCL stem segments - were recorded (Figures 3.39 and 3.40). The TCL stem segments showed a significant increase in secondary metabolite concentrations compared to the stem segments. Specifically, compounds like gallic acid, catechin, chlorogenic acid, EGCG, rutin, ellagic acid (EA), quercetin, apigenin, and quercitrin all showed higher concentrations in the TCL stem segments. These increases reflect an overall rise in phenolic and flavonoid compounds during the culture process, with changes in concentrations from initial to final levels, clearly illustrating the transformation in secondary metabolite formation (Figure 3.39).

Moreover, the levels of two important secondary metabolites, hypophyllanthin and phyllanthin, in adventitious roots from TCL stem segments (11.54 and 16.01 µg/g, respectively) were significantly higher than those from stem segments (3.99 to 9.03 µg/g) after 60 days of culture (Figure 3.40).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Nội dung 1: Study on selecting the optimal explant source for in vitro morphological development in passion fruit, gerbera, and P. amarus

The study results indicate that the position of the explant on the stem and the age of the explant have a significant impact on the morphological regeneration potential, such as shoots, somatic embryos, callus, and adventitious roots. In passion fruit, the strongest ability to regenerate adventitious shoots was observed at the third stem node. For gerbera, the flower bud stage was identified as the ideal time for somatic embryo regeneration. In the case of *P. amarus*, the second stem node optimized callus formation, while the third stem node promoted the formation of adventitious roots.

The TCL technique has been shown to be more effective than traditional culture methods. In this study, TCL explants enhanced the regeneration rates and quality of shoots, somatic embryos, callus, and adventitious roots in all three species.

Content 2: Study on the correlation between endogenous hormone levels during morphological development in passion fruit, gerbera, and P. amarus

Studies on the dynamics of endogenous hormones during adventitious shoot formation, callus induction, and adventitious root development in gerbera and Phyllanthus have demonstrated that plant hormones such as IAA, CKs, GA_3, ABA, MEL, and SA play crucial roles in regulating physiological and biochemical processes related to plant morphogenetic capacity. The results indicate that changes in the ratios among these hormones throughout the culture period directly affect cell division as well as the formation of callus tissue, somatic embryos, adventitious shoots, and adventitious roots.

In passion fruit, the interaction between IAA and CKs determines the efficiency of adventitious shoot regeneration. In gerbera, CKs and GA_3 are the

dominant hormones associated with somatic embryogenesis. In *P. amarus*, IAA and ABA govern callus formation and adventitious root development. The lTCL technique contributes to optimizing the balance of endogenous hormones, thereby enhancing morphogenetic efficiency compared with conventional explants.

Content 3: Study on the effect of the thin cell layer culture technique on antioxidant enzyme activity during shoot formation in passion fruit, somatic embryo formation in gerbera, and adventitious root formation in P. amarus; secondary metabolite accumulation in adventitious roots of P. amarus

The results indicate that the TCL culture technique has a pronounced effect on ET accumulation during *in vitro* morphogenesis. Compared with conventional explants, TCL explants exhibited higher ET accumulation and showed a strong correlation with morphogenetic efficiency, including adventitious shoot regeneration in passion fruit, somatic embryo formation in gerbera, and adventitious root development in Phyllanthus. Ethylene functions not only as a byproduct of culture-induced stress but also as a regulator of cell division and differentiation, interacting with other endogenous hormones at different stages of morphogenesis.

In addition, the TCL technique contributes to the modulation of antioxidant system activity, as evidenced by increased activities of the enzymes SOD, CAT, and APX, as well as changes in the levels of non-enzymatic antioxidant compounds. These findings suggest that TCL induces a controlled physiological stress state, thereby simultaneously promoting morphogenetic processes and enhancing the physiological adaptability of *in vitro* cultured tissues.

Recommendations

Within the defined scope and limitations of this study, the research was limited to an initial assessment of the correlation between endogenous hormones

and morphogenetic processes. Therefore, to achieve more in-depth, comprehensive studies with greater applicability, the following recommendations are proposed:

Further investigate the relationships among endogenous hormones during morphogenesis in different plant species.

Examine the correlations between endogenous hormone levels and genes involved in hormone biosynthesis, as well as genes associated with morphogenetic processes.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

[1] Nguyen Thi Nhu Mai, Truong Hoai Phong, Hoang Dac Khai, Do Manh Cuong, Vu Quoc Luan, Hoang Thanh Tung, Pham Thi Minh Thu, Hoang Thi Nhu Phuong, Nguyen Quang Vinh, Duong Tan Nhut, Endogenous hormone alteration during callus and adventitious root formation through thin cell layer culture system in *Phyllanthus amarus*. Plant Cell, Tissue and Organ Culture, 2024, 159(2), 1-18. https://doi.org/10.1007/s11240-024-02913-3

[2] Nguyen Thi Nhu Mai, Truong Hoai Phong, Do Manh Cuong, Vu Quoc Luan, Hoang Thanh Tung, Hoang Thi Nhu Phuong, Nguyen Quang Vinh, Hoang Hai Dang, Duong Tan Nhut, The changes of ethylene gas accumulation, antioxidant system activity, and secondary metabolite synthesis during *in vitro* adventitious root formation of *Phyllanthus amarus*. Plant Cell, Tissue and Organ Culture, 2025, 160(1), 11. https://doi.org/10.1007/s11240-024-02948-6