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EFFECTS OF SOME FACTORS ON THE FLOWERING AND INITIAL FRUITING OF PURPLE PASSION FRUIT (*Passiflora edulis* Sims f. *edulis*) CULTURED *IN VITRO*

SUMMARY OF DISSERTATION ON PLANT PHYSIOLOGY

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INTRODUCTION

Knowledge about the flowering of plants provides the theoretical foundation for developing appropriate methods to study plant physiology and selecting suitable hybrids in breeding strategies. Understanding the mechanisms and factors affecting flowering contributes to optimizing flowering time, number of flowers, pollination and seed production in plants.

However, the natural flowering of plants is generally restricted by season and this process is also significantly influenced by many environmental factors. Under these circumstances, the biotechnology approaches can contribute to overcome these limitations. The *in vitro* flowering system is considered as a convenient tool to study flower induction, flower senescence and flower organ development. This technique facilitates the understanding of flowering and fruiting physiology by controlling the influence of factors such as light, temperature, plant growth regulators, and minerals. *In vitro* flowering studies has a great potential in breeding programs for crop improvement based on the advantage of shortening and synchronization of the flowering time. If the *in vitro* flowering process is well described, it can serve as a model system for studying flowering mechanisms. *In vitro* flowering was commonly reported in many plant groups such as flowers and vegetables.

On the other hand, *Passiflora* is the largest genus in the family Passifloraceae. Among them, purple passion fruit (*Passiflora edulis* Sims f. *edulis*) is one of the species that brings significant value both commercially and medicinally. Based on published reports, there is no record of *in vitro* flowering of purple passion fruit. Furthermore, *in vitro* flowering is not a common phenomenon in the genus *Passiflora*, only flowering in *P. suberosa* L. has been recorded under *in vitro* conditions. Therefore, studying flowering *in vitro* is important in creating a foundation for further understanding flowering in this genus. Therefore, research into *in vitro* flowering control

on purple passion fruit plants is a necessary and highly applicable research direction in many aspects. Besides, purple passion fruit is a species with bisexual flowers. Based on the flower's self-pollination characteristics, research on *in vitro* fruiting is highly feasible.

Hence, the study on "Effects of some factors on the flowering and initial fruiting of purple passion fruit (*Passiflora edulis* Sims f. *edulis*) cultured *in vitro*" opens up a new potential direction on this plant.

Research objectives: Determine the effects of some factors including PGR (gibberellic acid A_3 - GA_3, abscisic acid - ABA), metallic salts and nanoparticles (silver nitrate - $AgNO_3$, silver nanoparticles - AgNPs, cobalt nanoparticles - CoNPs), and polyamine (spermidine - Spd) on *in vitro* growth, flowering and initial fruiting of purple passion fruit.

Research subjects: The growth, flowering, and initial fruiting of purple passion fruit (*Passiflora edulis* Sims f. *edulis*) under the influence of some additional factors on the culture media, including GA_3, ABA, $AgNO_3$, AgNPs, CoNPs, and Spd under *in vitro* conditions.

New contributions of the thesis: (1) The research has contributed to significantly improving the efficiency of shoot regeneration and somatic embryogenesis of purple passion fruit. (2) The research results provided reliable information about the impact of factors such as PGR, metal nanoparticles, and PA on growth, flowering, and initial fruiting from purple passion fruit shoots under *in vitro* conditions. (3) The results provided a reference process for applying *in vitro* flowering and fruiting based on the impact of AgNPs, thereby creating a foundation for further research.

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

Chapter 1. OVERVIEW

1.1. Flowering and *in vitro* flowering in plants

1.1.1. The roles of flowering and in vitro flowering

1.1.2. The main stages of flowering

1.1.3. Flowering pathways

1.1.4. Flower development models

1.2. Several factors affect flowering in plants

1.3. Some studies on flowering and fruit production *in vitro*

1.4. Overview of the purple passion fruit

1.4.1. Introduction

1.4.2. Some studies on purple passion fruit under in vitro conditions

1.4.3. Flowering and fruiting of purple passion fruit

In the genus *Passiflora*, flowering is not a common phenomenon in *in vitro* culture. According to current reports, *in vitro* flowering has only been recorded in *P. suberosa*. In this report, *P. suberosa* cultured for 21 days on MS medium supplemented with 3% sucrose, glycine, vitamins and cytokinin flowered *in vitro*. The study also showed that *in vitro* flowering depends on the location and origin of the explant. Leaf and internode explants flowered only if they originate near the tips; explants originating below the 5th node produced only non-flowering shoots. Besides, most flowers formed *in vitro* lack stamens; only a few complete flowers are produced. This study also showed that *P. caerulea*, *P. edulis* Sims., *P. foetida* and *P. trifasciata* only produced buds but did not flower when cultured under similar *in vitro* conditions. In general, in the field of *in vitro* flowering, research on the genus *Passiflora* is still very limited. Based on current reports, there are no reports of *in vitro* flowering of purple passion fruit (*P. edulis* Sims f. *edulis*), one of the commercially valuable species in this genus.

Chapter 2. CONTENTS, MATERIALS, AND METHODS

2.1. Content of research

Content 1: Research production of *in vitro* explants of purple passion fruit.

Content 2: Investigate the effects of some factors on the flowering and initial fruiting of purple passion fruit under *in vitro* culture conditions.

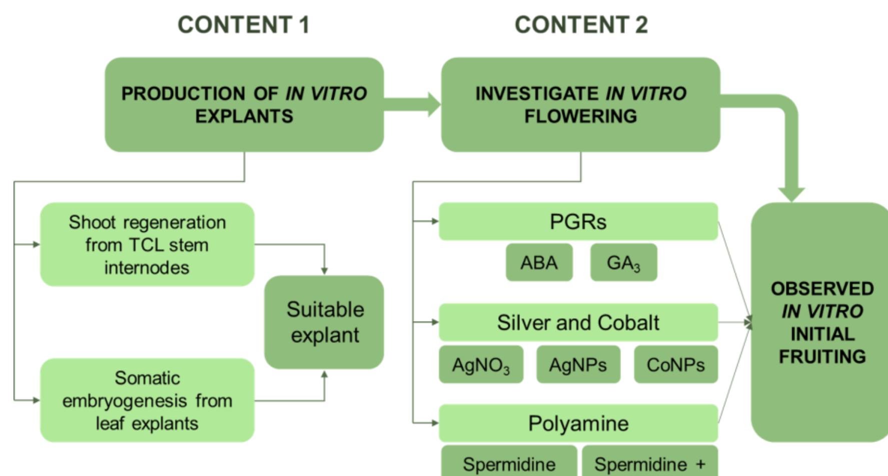

Figure 2.1. The diagram shows an overview of the research contents.

2.2. Research materials

2.2.1. Plant materials

In content 1, leaf and stem internode samples of 6-month-old purple passion fruit (*Passiflora edulis* Sims f. *edulis*) at the nursery of the Taynguyen Institute for Scientific Research were used as initial materials. In content 2, the apical shoots of *in vitro* purple passion fruit plants were used to arrange flowering experiments. The explants in each experiment were described specifically in the Research Methods section.

2.2.2. Equipment, instruments and chemicals

2.2.3. Culture media

2.3. Research methods

2.3.1. Experimental arrangement method

2.3.1.1. Content 1: Research on production of in vitro explant sources in purple passion fruit

- Experiment 1: Research on shoot regeneration from *ex vitro* TCL explants from stem internodes

Experiment 1.1. Investigating the influence of stem internode position on shoot induction: *ex vitro* stem internode segments (1 cm) at the 1st to 5th internode positions (from the shoot tip) were cut transversely with a thickness of about 0.2 cm to create tTCL explants. tTCL explants were cultured on MS medium supplemented with 1.5 mg/L BA and 1.0 mg/L NAA to investigate shoot induction.

Experiment 1.2. Investigating shoot induction from TCL stem internode explants: In this experiment, *ex vitro* stem internode segments at the appropriate internode position for shoot generation (surveyed in the experiment above) were used as sample sources. Stem internode segments (1 cm) were cut transversely into 5 tTCL explants or longitudinally into 4 lTCL explants. The explants were grown on MS medium supplemented with 1.5 mg/L BA; 1.0 mg/L NAA to compare shoot induction efficiency.

Experiment 1.3. Effect of AgNPs on shoot regeneration from TCL explants: Similar to establishing lTCL explants, stem internode segments with a length of 1 cm and a diameter of about 0.4 cm were cut longitudinally into 4 explants, then removed the inner part and retained only the outer cell layers (approximately 0.1 cm thick) to form the oTCL explant. lTCL and oTCL cultures were grown on MS medium supplemented with 1.5 mg/L BA; 1.0 mg/L NAA and AgNPs (0, 1.0, 3.0, 5.0, and 7.0 mg/L) at different concentrations to investigate and improve the shoot induction efficiency.

• Experiment 2: Research on somatic embryogenesis from *ex vitro* leaf explants of purple passion fruit

Experiment 2.1. Effects of 2,4-D and NAA on somatic embryogenesis: *Ex vitro* leaf explants (1.0 × 1.0 cm) were used as explants. In this experiment, explants were cultured on MS medium containing 2,4-D (0, 1.0, 2.0, 3.0 and 4.0 mg/L) or NAA (0, 1.0, 2.0, 3.0 and 4.0 mg/L) to investigate somatic embryo (SE) induction.

Experiment 2.2. Effect of auxin combined with AgNPs on somatic embryogenesis: To study the effect of AgNPs on SE, leaf explants (1 × 1 cm) were cultured on MS medium supplemented with PGRs (studied in the above experiment) and AgNPs at different concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L).

- Experiment 3: Research on rapid multiplication of suitable explants: Regenerated shoots from the optimal treatment explant source (surveyed in previous experiments) were selected and transferred to the culture medium. Shoots with a length of about 1 cm were collected and cultured on MS medium supplemented with 1.0 mg/L meta-Topolin (mT) and AgNPs at different concentrations (0, 1.0, 3.0, 5.0 and 7.0 mg/L).

2.3.1.2. Content 2: Investigate the effects of some factors on the flowering and initial fruiting of purple passion fruit under in vitro culture conditions

Experimental materials: Passion fruit shoots (from the appropriate regeneration process surveyed above) were multiplied in the optimal medium investigated in Content 1. After regeneration, the shoots were transferred to MS medium concluding with 2.5 mg/L IBA to stimulate root formation within 60 days. Shoot tips (from shoots that have formed roots) with a height of about 1.5 cm (including 1 tip and 3 leaves) were used as a source of explants for *in vitro* flowering and fruiting experiments (Fig. 2.5).

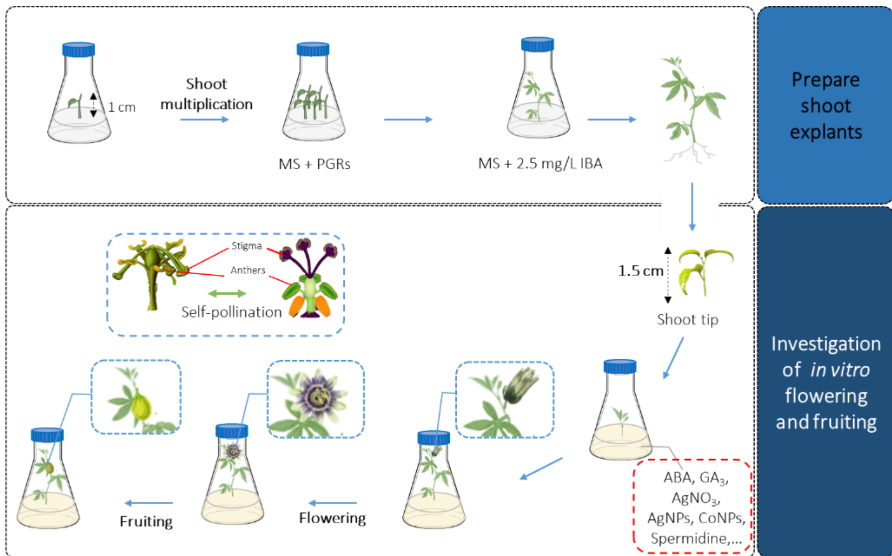

Figure 2.5. The schematic diagram of the experimental design

• Experiment 1. Investigate the effects of exogenous PGR on *in vitro* flowering and fruiting

Experiment 1.1. Investigating the effects of GA_3 on *in vitro* growth, flowering, and fruiting: *In vitro* passion fruit shoots about 1.5 cm tall were transferred to MS medium containing 30 g/L sucrose and 8 g/L agar and supplemented with different concentrations of GA_3 (0, 1.0, 1.5, 2.0, 3.0 mg/L). After the MS medium was autoclaved, GA_3 was filtered through a sterilized filter and cold added to the culture medium.

Experiment 1.2. Investigating the effects of ABA on *in vitro* growth, flowering, and fruiting: *In vitro* shoots about 1.5 in tall were transferred to MS medium containing 30 g/L sucrose and 8 g/L agar and ABA supplementation at different concentrations (0, 1.0, 1.5, 2.0, 3.0 mg/L).

• Experiment 2. Investigate the effects of silver and cobalt on *in vitro* growth, flowering and fruiting

Experiment 2.1. Investigating the effects of $AgNO_3$ on *in vitro* growth, flowering and fruiting: Shoot tips with a length of about 1.5 cm were cultured on MS medium, 30 g/L sucrose, 8 g/L agar, and supplemented with $AgNO_3$ (0, 1.0, 3.0, 5.0, 7.0, and 9.0 mg/L) at different concentrations to investigate *in vitro* flowering.

Experiment 2.2. Investigating the effects of AgNPs on *in vitro* growth, flowering and fruiting: Shoot tips with a length of about 1.5 cm were cultured on MS medium, 30 g/L sucrose, 8 g/L agar, and supplemented with AgNPs (0, 1.0, 3.0, 5.0, 7.0, and 9.0 mg/L) at different concentrations to investigate *in vitro* flowering. Treatments without AgNPs were used as controls.

Experiment 2.3. Investigating the effects of CoNPs on *in vitro* growth, flowering and fruiting: Shoot tips with a length of about 1.5 cm were transferred to basal MS medium (or MS medium removed $CoCl_2$ salt), containing 30 g/L sucrose, 8 g/L agar, and supplemented with CoNPs at different concentrations (0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/L) to investigate *in vitro* flowering.

• Experiment 3. Investigate the effects of polyamine on growth, flowering and fruiting *in vitro*

Experiment 3.1. Investigating the effects of spermidine (Spd) alone on *in vitro* growth, flowering and fruiting: *In vitro* shoots about 1.5 cm tall were transferred to MS medium supplemented with Spd concentrations (0, 0.05, 0.1, and 0.2 mM) to investigate *in vitro* flowering. After the MS medium was autoclaved, Spd was filtered through a sterilized filter and added to the culture medium.

Experiment 3.2. Investigating the effects of combined spermidine on *in vitro* growth, flowering and fruiting: Similar to the previous experiment,

in vitro shoots about 1.5 cm tall were cultured on MS medium supplemented Spd concentrations (0, 0.05, 0.1, 0.2, and 0.3 mM) combined with appropriate factors (were investigated in the above experiment) to enhance *in vitro* flowering.

- Arrange and monitor *in vitro* flowering and fruiting

For flowering experiments: Each culture flask was arranged with 1 shoot. Each treatment was conducted with 60 culture flasks and repeated 3 times.

Characteristics of flowering and fruiting during this process were analyzed and observed according to the stages of development. The flowering and fruiting characteristics of 2-year-old plants *ex vitro* were used to compare with plants under *in vitro* conditions.

2.3.2. Methods and techniques used in research

2.3.2.1. Collect and evaluate monitoring indicators in the research

2.3.2.2. Quantification of endogenous hormones

Gibberellic acid (GAs), abscisic acid (ABA) and melatonin contents of shoots were determined by HPLC method after 60 days of culture.

2.3.2.3. Quantification of accumulated ethylene gas content in culture flasks

Gas chromatography with a flame ionization detector was used to quantify ethylene gas accumulated in the flask after 60 days of culture.

2.3.2.4. Histological analysis

Cytological changes during *in vitro* shoot and flower formation were monitored by anatomical methods. Sample observation was conducted on an optical microscope with a 10× eyepiece, and 10× and 40× objectives.

2.3.2.5. Statistical analysis

Data obtained from the experiments were analyzed using SPSS 26.0 statistical software (with appropriate tests for each experiment) with a significance level of $p < 0.05$. Use Microsoft Excel 2019 software to draw graphs and represent statistical results.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Content 1: Research production of *in vitro* explants of purple passion fruit

3.1.1. Research on shoot regeneration from ex vitro TCL explants from internodes

Overall, for *ex vitro* TCL explants from stem internodes, optimal shoot induction (70.37%) was observed in explants at the 3rd internode position. The shoot induction rate of tTCL and lTCL at the 3rd internode position were not significantly different; however, the number of shoots in lTCL was significantly higher than that in tTCL. The oTCL explants showed a higher shoot regeneration rate than the lTCL explants. The addition of AgNPs at appropriate concentrations on the culture medium also significantly improved the shoot regeneration efficiency of lTCL (5.0 mg/L AgNPs) and oTCL (3.0 mg/L AgNPs) explants.

3.1.2. Research on somatic embryogenesis from ex vitro leaf explants

The addition of AgNPs at all experimental concentrations resulted in a higher rate of somatic embryogenesis and the number of SE per explant compared to the control. In particular, the supplemented culture medium combined with 2.0 mg/L 2,4-D and 2.0 mg/L AgNPs resulted in SE induction rate (92.59%) and number of SE per explant (31.67 embryos) optimally. In addition, embryogenic callus formation and SE were observed on medium supplemented with AgNPs more clearly than the control after 15 days, 30 days, and 75 days. In addition, all experimental treatments showed the ability to form plantlets after 120 days of culture. The addition of 2.0 mg/L 2,4-D combined with different concentrations of AgNPs resulted in significantly higher numbers of plantlets formed from SE than the addition of 2,4-D alone. In particular, adding 2.0 mg/L 2,4-D combined with 2.0 mg/L AgNPs gave the highest number of plantlets (6.67 plants/explant).

Based on the effectiveness of plant regeneration, it can be seen that shoot regeneration from oTCL explants was more effective than SE in terms of the number of explants as well as the average time of the process. For oTCL explant, the average number of regenerated shoots after 60 days of culture was 15.33 shoots/explant; while an average of 6.67 plantlets formed from SE after 120 days of culture. Hence, regenerated shoots from oTCL explants were selected as the initial explant source for the following experiments.

3.1.3. Effect of AgNPs on shoot multiplication

The results showed that the addition of AgNPs on the culture medium significantly enhanced the shoot multiplication efficiency. The treatment supplemented with 5.0 mg/L AgNPs had the highest number of shoots (13.67 shoots/explant) and shoot height (4.43 cm). Furthermore, the addition of AgNPs on the culture medium also significantly increased the SPAD index in leaves (33.93) compared to the control (22.18).

3.2. Content 2: Investigate the effects of some factors on the flowering and initial fruiting of purple passion fruit under *in vitro* culture conditions.

3.2.1. Effects of some exogenous PGRs on in vitro flowering

3.2.1.1. Effect of GA_3 on growth and flowering in vitro

In this study, preliminary results showed that *in vitro* flowering induction in purple passion fruit plants was not observed on medium supplemented with GA_3 alone within the limits of the experiment.

3.2.1.2. Effect of ABA on growth and flowering in vitro

Overall, preliminary results showed reduced shoot growth on ABA-supplemented medium, but did not induce *in vitro* flowering of purple passion fruit plants within the limits of the experiment.

3.2.2. The effects of silver and cobalt on in vitro growth, flowering and fruiting

3.2.2.1. Effect of $AgNO_3$ on growth and flowering in vitro

Preliminary results showed that *in vitro* flowering induction in purple passion fruit plants was not observed on medium supplemented with $AgNO_3$ alone at the experimental concentrations.

3.2.2.2. Effect of AgNPs on growth and flowering in vitro

After 60 days of culture, the growth of shoots was significantly enhanced on culture medium supplemented with AgNPs at appropriate concentrations. The highest shoot height (7.50 cm) was recorded at the concentration of 7.0 mg/L AgNPs and was significantly higher than the control (2.07 cm). Treatment supplemented with 3.0 mg/L AgNPs significantly increased the number of leaves (13.33 leaves/shoot) and SPAD index (30.12) compared to the control (6.67 leaves/shoot and 27.12; respectively). However, the SPAD index decreased significantly when increasing the concentration of additional AgNPs to 7.0 and 9.0 mg/L compared to the control (Table 3.6).

Table 3.6. Effects of AgNPs on *in vitro* growth and flowering after 60 days of culture

AgNPs (mg/L)	Shoot height (cm)	No. of shoots /explant	SPAD	Flowering rate (%)	No. of flower buds/shoot
0	2.07^{e*}	6.67^{d}	27.12^{bc}	0.00^{e}	-
1.0	3.50^{d}	10.67^{c}	28.40^{ab}	0.00^{e}	-
3.0	7.23^{a}	13.33^{ab}	30.12^{a}	11.58^{d}	0.67^{bc}
5.0	6.27^{c}	10.67^{c}	25.68^{cd}	23.60^{c}	1.00^{b}
7.0	7.50^{a}	12.00^{bc}	24.73^{d}	51.72^{a}	2.33^{a}
9.0	6.77^{b}	14.67^{a}	24.00^{d}	38.20^{b}	1.33^{b}

** In the same column, values followed by the same letter (a, b, ...) represent statistically insignificant differences at $p < 0.05$ (Duncan's test).*

On the other hand, *in vitro* flowering was observed in shoots cultured on medium supplemented with 3.0 to 9.0 mg/L AgNPs with flowering rates ranging from 11.58% to 51 72% after 60 days of culture (Table 3.6, Figure 3.14A). Among them, shoots cultured on a medium supplemented with 7.0 mg/L AgNPs showed the highest flowering rate (51.72%) and number of flowers (2.33 flowers/shoot). The flowering rate and number of flowers decreased significantly when increasing the concentration of AgNPs supplemented on the culture medium to 9.0 mg/L (38.20% and 1.33 flowers/shoot, respectively).

Additionally, shoots cultured on medium supplemented with 7.0 mg/L AgNPs showed significantly lower levels of endogenous GAs (94.146 µg/g) and ABA (1.498 µg/g) compared to the control (141.354 µg/g and 2.006 µg/g, respectively) after 60 days of culture. In this study, melatonin content in shoots cultured in the treatment supplemented with 7.0 mg/L AgNPs (0.229 µg/g) was also significantly lower than the control (0.383 µg/g) (Figure 3.14 B).

In addition, the ethylene content in the culture flask in the treatment supplemented with 7.0 mg/L AgNPs (0.1262 ppm) was also higher than the treatment without AgNPs (0.0696 ppm) (Fig. 3.14B). However, the higher accumulation of ethylene may be due to the high concentration of added AgNPs and the physiological stage of the plants. Therefore, ethylene accumulation and its impact on flowering in purple passion fruit need to be investigated and clarified in future studies. On the other hand, observation results show that flower buds were induced at the position under the leaf axils at the nodes close to the tips. In shoots that were not induced to flower, vegetative buds were formed and developed at the stem nodes (Fig. 3.15). After 40 - 45 days of culture under *in vitro* conditions, anatomical results showed a transition from vegetative buds to flower buds on culture medium supplemented with 7.0 mg/L AgNPs. The flower buds have an enlarged

growth tip, bulging up in a dome shape and initially forming flowers (Fig. 3.17).

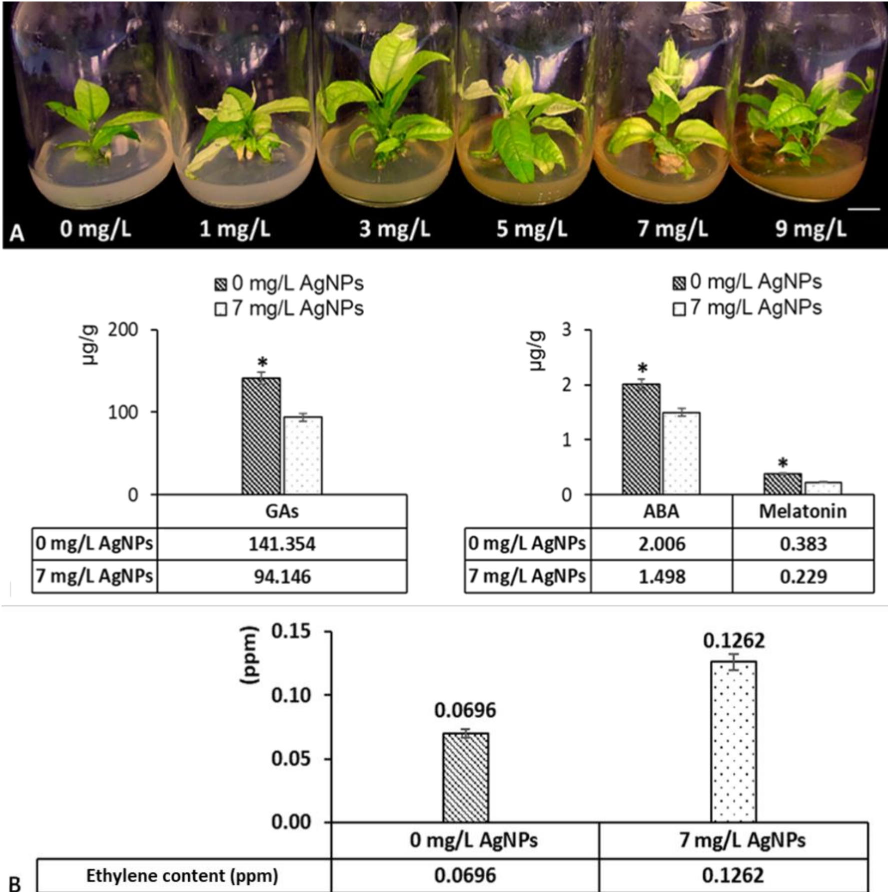

Figure 3.14. Effects of AgNPs on *in vitro* flowering, endogenous hormone changes and ethylene accumulation after 60 days of culture. **A.** *In vitro* flowering on medium supplemented with AgNPs at different concentrations (Bar: 1 cm). **B.** GAs, ABA, melatonin content of shoots and accumulated ethylene content in the culture flask in the treatment supplemented with 7.0 mg/L AgNPs and the control.

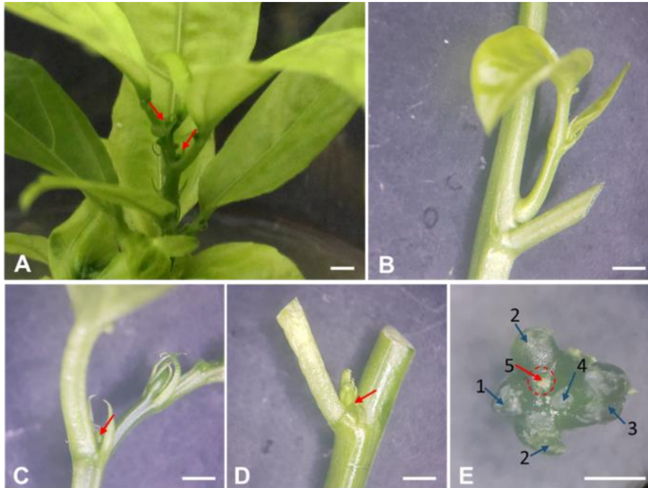

Figure 3.15. *In vitro* flower bud formation and development on medium supplemented with 7.0 mg/L AgNPs. **A.** Location of flower bud formation. **B.** Vegetative buds develop when flower buds do not form. **C.** Formation of flower buds at the shoot tips after 45 days of culture. **D.** and **E.** Formation of flower buds at stem nodes after 45 days of culture (Bar: 1 cm). *(The red arrow indicates the location of the flower bud)*.

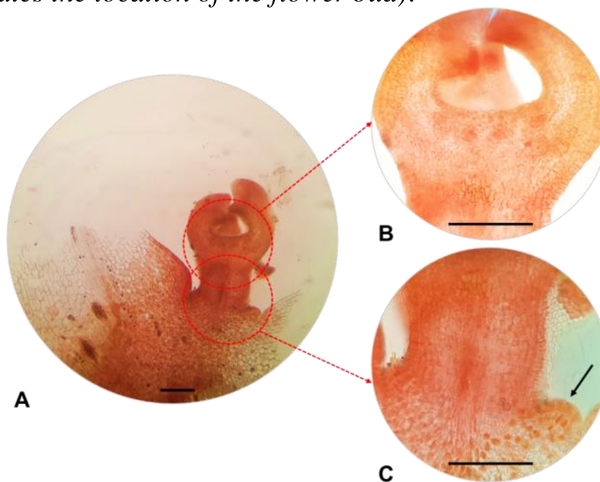

Figure 3.17. Flower bud anatomy from *in vitro* shoots cultured on medium supplemented with 7.0 mg/L AgNPs after 45 days of culture. **A.** Flower buds. **B.** Expanded meristem region of floral primordia. **C.** Tissue forming tendril (Bars: 40 µm).

The results also showed that the bloom rate (23.28 - 100%) was observed in the AgNPs-supplemented treatments at the 70th day of culture. The highest bloom rate (100%) was recorded in the 7.0 mg/LAgNPs treatment; However, when the AgNPs concentration increased to 9.0 mg/L, the bloom rate decreased sharply (63.14%). In addition, the flowers with the largest diameter (3.43 cm) were also observed in the treatment supplemented with 7.0 mg/L AgNPs (Fig. 3.18).

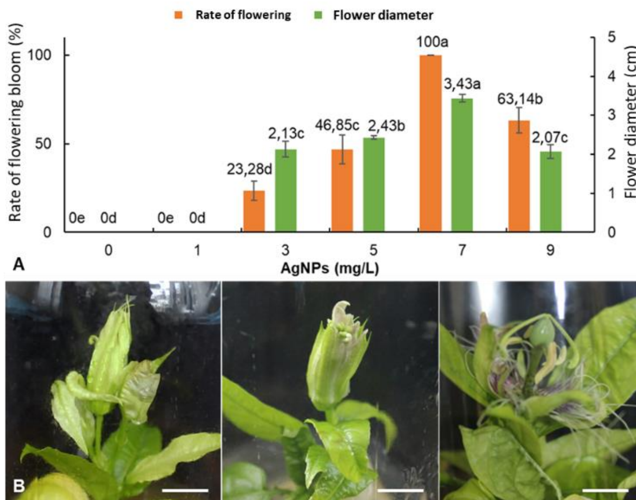

Figure 3.18. Effect of AgNPs on *in vitro* flowering after 70 days of culture. **A.** Bloom rate and flower diameter on culture medium supplemented with different concentrations of AgNPs. **B.** Blooming on medium supplemented with 7.0 mg/L AgNPs (Bars: 1 cm).

On the other hand, a comparison with natural flower buds from 2-year-old plants showed that *in vitro* flower buds were small in size and the surrounding sepals were thinner. In addition, most *in vitro* flowers often lack bracts or the bracts fall during flower bud development (Fig. 3.19). Some strictly regulated developmental programs in plants can be compromised by mutations or by environmental signals. Therefore, the phenomenon noted above needs to be further clarified in further studies.

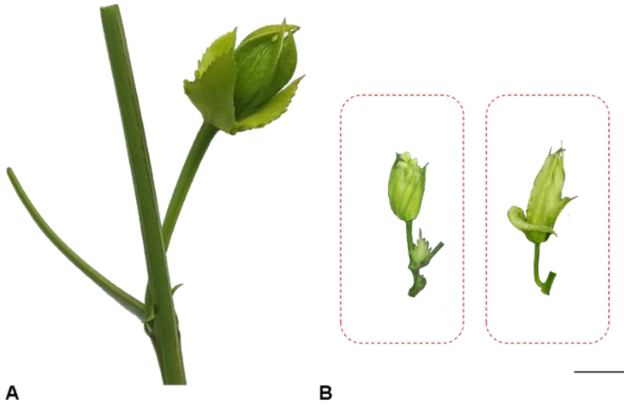

Figure 3.19. Characteristics of natural flower buds and *in vitro* flower buds. A. Natural flower buds from a 2 years old plant. B. *In vitro* flower buds from apical buds after 60 days of culture on medium supplemented with 7.0 mg/L AgNPs. (Bar: 1 cm).

Figure 3.20. Some characteristics of natural passion fruit flowers from 2-year-old plants and flowers from *in vitro* buds (Bars: 1 cm).

In addition, when comparing *in vitro* flowers and natural flowers, many different characteristics are revealed. *In vitro* flowers was smaller in size, and some *in vitro* flowers had unusual characteristics compared to natural flowers. Natural flowers consist of five stamens with large anthers containing pollen. A pistil has three styles extending from the ovary, each branch ending in a stigma (Figure 3.20). For *in vitro* flowers, some typical aberrations such as paucity of pollen grains, disappearance of petals, lack of a style or stamen, or underdevelopment of anthers have been observed (Fig. 3.20).

3.2.2.3. Effect of AgNPs on in vitro fruiting

Results showed that most *in vitro* flowers have reproductive organs such as ovaries, stigmas, and anthers that were capable of forming young fruits after 90 days of culture (Fig. 3.21). Flowers with stamen abnormalities are often unable to produce fruit *in vitro*. Flowers that do not produce fruit show signs of wilting about 10 days after blooming (Figure 3.21B). The fruit formation rate in the treatment using AgNPs at appropriate concentrations was significantly higher than the control. Among them, the treatment using 7.0 mg/L AgNPs had the highest fruiting rate (56.67%), number of fruits (1.67 fruits), and fruit diameter (1.13 cm) (Table 3.7).

Table 3.7. Effects of AgNPs on *in vitro* fruiting after 90 days of culture

AgNPs (mg/L)	Fruiting rate (%)	No. of fruits /shoot	Diameter of young fruit (cm)
0	0.00^{d*}	0.00^{c}	0.00^{d}
1.0	0.00^{d}	0.00^{c}	0.00^{d}
3.0	20.09^{c}	1.00^{b}	0.07^{c}
5.0	35.82^{b}	1.00^{b}	0.77^{bc}
7.0	56.79^{a}	1.67^{a}	1.13^{a}
9.0	33.53^{b}	1.00^{b}	0.83^{bc}

** In the same column, values followed by the same letter (a, b, ...) represent statistically insignificant differences at $p < 0.05$ (Duncan's test).*

Figure 3.21. *In vitro* flowering and fruiting on medium supplemented with 7.0 mg/L AgNPs. **A.** Flowers have complete stamens and pistils. **B.** Flowers that did not produce fruit wilted after 10 days of blooming. C. Young fruits formed after 20 days of blooming (Bars: 1 cm). *(The red arrow indicates the location of the flower, and the green arrow indicates the location of the fruit).*

3.2.2.4. Effect of CoNPs on growth and flowering in vitro

In this experiment, purple passion fruit shoots did not flower *in vitro* on medium supplemented with CoNPs at the experimental concentrations.

3.2.3. Effects of polyamine on growth, flowering and fruiting in vitro

The results showed that shoot height was significantly improved on medium supplemented with Spd after 90 days of culture. Specifically, Spd at concentrations from 0.05 - 0.2 mM gave shoot height (5.77 - 8.87 cm) significantly higher than the control treatment (5.13 cm). The highest number of leaves per shoot (10.33 leaves) was recorded on medium supplemented with 0.1 mM Spd. In addition, the results also showed that the SPAD index increased significantly with 0.1 mM and 0.2 mM Spd (33.75 and 32.94; respectively). However, when increasing the Spd concentration to 0.3 mM, the results recorded a significant decrease in both shoot height and leaf SPAD index.

In previous experiments, *in vitro* flowering in purple passion fruit was observed to be optimal on medium supplemented with 7.0 mg/L AgNPs. In this experiment, different concentrations of Spd were added in combination with 7.0 mg/L AgNPs to investigate *in vitro* flowering. The results showed that combining Spd and AgNPs significantly affected the growth and flowering. After 90 days of culture, shoot height was significantly enhanced on medium supplemented with Spd at different concentrations. The highest shoot height (9.17 cm) was recorded in the treatment supplemented with 0.3 mM Spd. Treatments supplemented with 0.05 and 0.1 mM resulted in high numbers of leaves per shoot (13.67 and 14.33 leaves, respectively). However, when increasing the Spd concentration to 0.2 and 0.3 mM, the number of leaves per shoot decreased significantly (8.00 and 8.33 leaves, respectively). On the other hand, *in vitro* flowering was only recorded for shoots on medium supplemented with a combination of 0.05 mM Spd and 7.0 mg/L AgNPs after 90 days of culture (with 17.78% flowering with 1.33 flower buds per shoot). Additional treatments combined with Spd concentrations from 0.1 - 0.3 mM did not record flowering during the survey period (Table 3.11).

Table 3.11. Effect of Spd on medium supplemented with AgNPs on *in vitro* flowering after 90 days of culture.

AgNPs (mg/L)	Spd (mM)	Shoot height (cm)	No. of leaves /shoot	Flowering rate (%)	No. of flower buds/shoot
7.0	0.05	8.03^{b*}	13.67^{a}	17.78	1.33
	0.1	7.90^{b}	14.33^{a}	0.00	-
	0.2	7.77^{b}	8.00^{b}	0.00	-
	0.3	9.17^{a}	8.33^{b}	0.00	-

** In the same column, values followed by the same letter (a, b, ...) represent statistically insignificant differences at $p < 0.05$ (Turkey's test).*

On the other hand, observation of some shoots in medium supplemented with 0.05 mM Spd and 7.0 mg/L AgNPs showed the appearance of tendrils after 60 days of culture. This was also the location where *in vitro* flower appeared simultaneously (Fig. 3.29, 3.31).

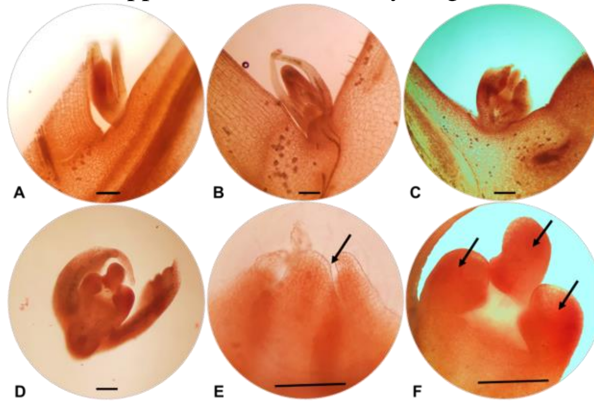

Figure 3.29. Anatomy of flower buds induced from *in vitro* shoots after 65 days of culture. **A** and **B.** Vegetative buds. **C - F.** Flower buds. (Bars: 40 µm).

Figure. 3.31. Flower bud formation of purple passion fruit *ex vitro* and *in vitro*. The top (**A**) and stem nodes (**B**, **C**) contain flower buds of the *ex vitro* plant. The top (**D**) and stem nodes (**E**, **F**) contain flower buds of the *in vitro* plant. (Bars 1 mm).

The recorded results show that most *in vitro* flowers have reproductive organs such as ovary, style, and anthers that are capable of pollination and forming young fruits. The flowers formed are capable of producing fruits *in vitro* (64.45%) and reach an average of 1.33 fruits/shoot after 120 days of culture (Fig. 3.32). When compared with the addition of 7.0 mg/L AgNPs alone, the addition of Spd combined with AgNPs showed better shoot growth than the addition of AgNPs alone. However, combined supplementation with Spd delayed flowering compared to individual AgNPs supplementation.

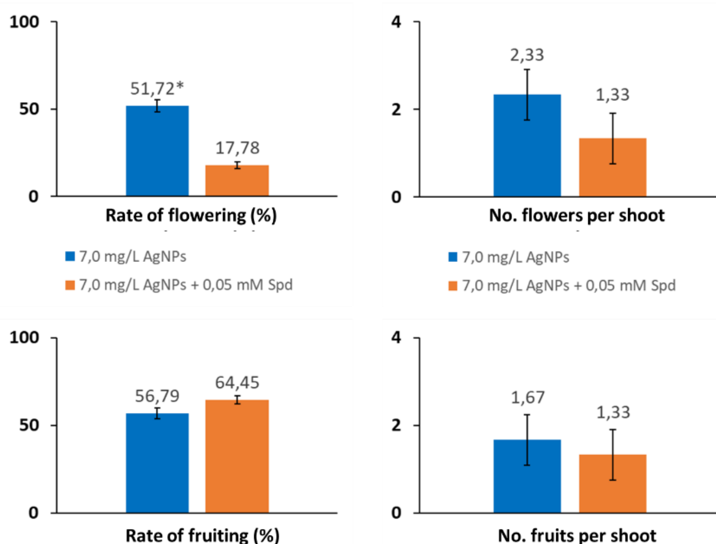

Fig. 3.32. Comparison of *in vitro* flowering and fruiting of purple passion fruit on medium supplemented with 7.0 mg/L AgNPs alone and in combination with 0.05 mM Spd.

The results indicated that flowering on medium supplemented with AgNPs combined with Spd was later than that on medium supplemented with AgNPs alone. However, the exact mechanism and interaction of AgNPs with Spd in this process needs to be clarified in further studies.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Content 1: Research on production of in vitro explant sources in purple passion fruit

The explant source from the shoot generation was more optimal than the explant source from the somatic embryogenesis based on regeneration efficiency.

Optimal shoot regeneration efficiency was recorded from oTCL cultures at the 3rd internode on MS medium supplemented with 1.5 mg/L BA; 1.0 mg/L NAA and 3.0 mg/L AgNPs.

MS medium supplemented with 1 mg/L mT and 3 mg/L AgNPs was suitable for shoot multiplication.

Content 2: Investigate the effects of some factors on flowering and initial fruiting of purple passion fruit under in vitro conditions

At appropriate concentrations, GA_3 enhanced shoot growth, while ABA reduced shoot growth at all supplementation concentrations. Media supplemented alone with ABA or GA_3 at experimental concentrations did not stimulate flowering of purple passion fruit shoots under *in vitro* conditions.

Silver in the form of NPs stimulated flowering from shoots of purple passion fruit plants, while in the form of $AgNO_3$ did not induce flowering within the limits of the study. The flowering rate ranged from 11.67% to 51.67% after 60 days of culture. *In vitro* flowering and fruiting significantly depended on the concentration of AgNPs. Some *in vitro* flowers have structural features that differ from those in nature. For CoNPs, shoots did not flower at all experimental concentrations after 60 days of culture.

In vitro flowering was not observed for shoots on medium supplemented with Spd alone at the experimental concentrations. The buds

flowered slowly, and the flowering rate and number of flowers decreased in the medium supplemented with Spd combined with AgNPs compared to the addition of AgNPs alone. The addition in combination with Spd gives the inflorescence structure close to that of natural inflorescences.

Recommendations

Continued research on *in vitro* flowering and fruiting of purple passion fruit from SE explants.

Research has shown that AgNPs have a positive effect on flowering and fruiting *in vitro*; However, to clarify these effects, it is necessary to continue research on the dynamic changes of endogenous hormones and especially the expression of related genes.

Further studies need to be performed to better understand the physiological characteristics as well as the factors that influence this process.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Truong Hoai Phong, Tran Hieu, Hoang Thanh Tung, Nguyen Thi Nhu Mai, Hoang Dac Khai, Do Manh Cuong, Vu Quoc Luan, Nguyen Ba Nam, Duong Tan Nhut, Silver nanoparticles enhance the *in vitro* plant regeneration via thin cell layer culture system in purple passion fruit. *Plant Cell, Tissue and Organ Culture*, **2023**, 155, 403-415. https://doi.org/10.1007/s11240-023-02566-8

2. Truong Hoai Phong, Tran Hieu, Hoang Thanh Tung, Nguyen Thi Nhu Mai, Hoang Dac Khai, Do Manh Cuong, Vu Quoc Luan, Nguyen Ba Nam, Duong Tan Nhut, Somatic embryogenesis as potential method for commercial propagation in *Passiflora edulis* Sims f. *edulis* – an important horticultural crop. *Scientia Horticulturae*, **2023**, 316, 112020. https://doi.org/10.1016/j.scienta.2023.112020

3. Truong Hoai Phong, Tran Hieu, Hoang Thanh Tung, Nguyen Thi Nhu Mai, Hoang Dac Khai, Do Manh Cuong, Vu Quoc Luan, Nguyen Ba Nam, Duong Tan Nhut, Silver nanoparticles: a positive factor for *in vitro* flowering and fruiting of purple passion fruit (*Passiflora edulis* Sims f. *edulis*). *Plant Cell, Tissue and Organ Culture*, **2022,** 151, 401-412. https://doi.org/10.1007/s11240-022-02361-x