Int. j. adv. multidisc. res. stud. 2025; 5(3):1570-1574

Received: 03-05-2025
Accepted: 13-06-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Factors Affecting the Success Rate in *in vitro* Grafting of Grapefruit Trees (*Citrus* spp.)

Le Thi Nhu Thao
Department of Agriculture and Food Technology, Tien Giang University, Vietnam

DOI: https://doi.org/10.62225/2583049X.2025.5.3.4492
Corresponding Author: **Le Thi Nhu Thao**

Abstract
Tissue culture is an effective technique in propagating disease-free citrus plants and conserving valuable varieties. This study aims to evaluate the factors affecting the success rate of *in vitro* grafting of grapefruit trees (Citrus spp.).) *in vitro*. The results show that Calcium hypochlorite treated for 10 minutes is the most effective disinfectant (100% sample cleanliness). The Rough Lemon rootstock has the highest compatibility rate with the Citrus reticulata buds (88.9%) and is suitable for many other bud varieties. Regarding the culture medium, a BA concentration of 0.1 mg/L resulted in the highest survival rates for all three bud varieties, with Da Xanh achieving 66.67% and Đường Lá Cam achieving 55.55%. The results provide a basis for the micrografting process applied in the propagation of disease-free grapefruit plants.

Keywords: Urinary Biomarkers, Exosomes, Biosensors, Liquid Biopsy, Multi-omics

1. Introduction

Citrus trees (Rutaceae family), especially pomelo varieties (Citrus spp.).), play an important role in agricultural production in Vietnam due to their high nutritional and economic value [1, 2]. However, the breeding of high-quality pomelo varieties is currently facing many challenges due to pests, especially viral diseases such as Greening, Tristeza, etc., which severely affect the yield and quality of the fruit [1, 3-5]. The application of disease-free breeding techniques is an essential requirement in the context of sustainable agricultural development [6-9].

Micrografting is one of the effective biotechnological solutions, allowing the regeneration of disease-free plants by combining healthy shoot tips with rootstocks that have been cultured under sterile conditions [10]. Compared to traditional breeding methods, micrografting helps shorten the breeding time, ensures genetic consistency, and increases the survival rate of the plants [11-14]. However, the effectiveness of this technique depends on many factors such as sterilization agents, root-shoot combinations, and the concentration of growth regulators in the culture medium [15].

In this study, we evaluate the factors affecting the success rate of micrografting several popular grapefruit varieties in Vietnam, thereby proposing an optimal micrografting procedure that can be applied in small and medium-scale seed production.

2. Materials and Methods

2.1 Experimental materials and conditions

Rootstocks: The varieties used include Rough Lemon, Da Xanh Grapefruit, Long Co Co Grapefruit, and Honey Orange. The rootstock seeds are peeled, sterilized with a 10% Calcium hypochlorite solution for 15 minutes, and then washed three times with sterile distilled water. The seeds are sown in test tubes containing MS solid medium that has been sterilized and incubated in the dark (25 ± 2°C) for 14–16 days before grafting.

Growth apex (grafting shoots): Shoots are obtained from sources such as: (i) field-grown plants, (ii) branches incubated under tissue culture conditions, (iii) shoots preserved in greenhouses, or (iv) shoots from previously induced tissue culture samples. The buds are sterilized with 10% Calcium hypochlorite for 10 minutes, then washed three times with sterile distilled water.

2.2 Method

Grafting is carried out under sterile conditions, under a magnifying glass. The rootstock is cut off at the roots and top, then a triangular grafting notch approximately 0.1–0.15 mm in size is created at the apex. The graft bud is precisely placed into this grafting groove. After grafting, the plant is maintained in a liquid MS medium, at 27°C, with a light intensity of 10,000 lux in the tissue culture room. In the first week, observe the graft union and remove any shoots arising from the rootstock to avoid competition for growth. After 45–60 days, the successfully grafted plants will develop 2–3 true leaves and be transferred to room temperature conditions for 2–4 days to acclimate before proceeding to the next treatment steps such as second grafting or plant training.

- **Experiment 1:** The effect of disinfectants on the survival and grafting compatibility of various pomelo cultivars.

The experiment was conducted to select disinfectants with appropriate concentrations and disinfection times for each type of variety to achieve the highest non-contamination and growth results. The experiment includes 15 treatments with: 2 types of disinfectants, namely Calcium Hypochlorite (10% concentration) for 10 minutes (according to the VNCCĂQMN procedure) and $HgCl_2$ at 0.05% and 0.01% for 5-10 minutes, and 3 bud varieties (Da Xanh, Đường Lá Cam, Long Cổ Cò grafted onto the same rootstock Rough RLMmon approximately 3 weeks old). Each treatment was repeated 3 times with 3 plants per repetition.

- **Experiment 2:** The effect of rootstock on grafting success rate and shoot growth.

The experiment was conducted on 4 types of rootstocks: Sweet orange (CM), Da Xanh grapefruit (DX), Long Co Co grapefruit (LCC), and Rough Lemon (RLM) to determine the grafting combinations (rootstock - scion) suitable for each type of grapefruit variety to achieve the highest grafting success rate and best growth. The seeds used for rootstock were collected from healthy mother trees with high yield and disease-free. The seeds are sown in a solid MS medium, and the rootstock plants that meet the standards for grafting are complete plants about three weeks old. The experiment was conducted with 12 treatments, each treatment with 3 replications, 3 plants/replication (LCC/RLM, ĐLC/RLM, DX/RLM, LCC/CM, ĐLC/CM). DX/CM, LCC/DX, ĐLC/DX, DX/DX, LCC/LCC, ĐLC/LCC, DX/LCC).

- **Experiment 3:** The effect of the growth regulator BAP on the grafting success rate and development of the grafted combinations of grapefruit varieties

The experiment determines the appropriate growth regulator for the graft union and development of the post-grafting combination. conducted with 3 types of buds (grapefruit: Long Co Co, Da Xanh, Duong La Cam), 1 type of rootstock Rough RLMmon, and the growth regulator BAP with 4 concentrations from 0 – 1 mg/l (0-1.0 mg/l). The experiment was conducted with 12 treatments, each treatment repeated 3 times, with 3 plants per repetition. The basic experimental environment is the MS liquid medium supplemented with the growth regulator BAP (0 – 1 mg/l).

The treatments with the same concentration of growth regulators must be monitored simultaneously to accurately assess and easily compare the differences in the degree of compatibility and development of various growth tips, thereby determining the appropriate concentration of growth regulators to optimize the grafting method for the highest efficiency.

2.3 Monitoring indicators

- **Experimental monitoring indicators 1:**

Infection rate (%) = (number of infected grafted plants/total number of grafted plants) x 100.

Non-infection rate (%) = (number of non-infected grafted trees/total number of grafted trees) x 100

Graft survival rate (%) = (number of grafted plants that survived, developed, and are disease-free/total number of grafted plants) x 100

- **Monitoring indicators for experiment 2:**

The union between the rootstock and the scion (union): "presence or absence of union" between the scion and the rootstock. This criterion is evaluated on the fifth day after grafting. Identifying characteristics:

+ The growth tip integrates well with the rootstock when the area just above the triangular graft is light green.

+ The growth apex that does not graft well will be brown or black (dead apex).

+ Note: It is necessary to distinguish between the grafted growth tip, which is light green, and the growth tip from the auxiliary bud, which is darker green. At this point, the grafted shoot tip may fall off due to the handling during the transfer to the test tube, or because the grafted tip fuses slowly while the lateral bud develops quickly, pushing the grafted tip out.

b) Survival and grafting

- The indicators and data are collected on the 10th day after grafting.
- The living apex and graft union have two buds developing out of the stem, and changes in size can be observed under fluorescent light with the naked eye.
- The growth apex during this stage can also die due to the rapid development of lateral buds or due to a weak graft base.

c) Survive grafting and develop into a complete graft bud (survive grafting and develop)

- The indicators and data are collected on the 15th to 20th day after grafting.
- The growth apex begins to develop strongly, with two cotyledons emerging from the stem, starting to develop into complete leaves.
- It is necessary to actively prune the lateral shoots to avoid nutrient competition between the lateral shoots and, and the grafted shoots.
- Experimental monitoring indicators 3:

The experiment was monitored with the following indicators:

- The survival rate and integration of the growth apex (evaluation and data collected on the 10th day post-grafting)
- The development in size of the growth apex observed visually under a fluorescent lamp compared to the control (criteria and data determined from the 15th day post-grafting).
- The size of the two cotyledons and the size of the growth apex.

2.4 Data collection and processing

The experiments were arranged completely randomly. The data are presented in statistical form and processed using IRRISTAT software. 3.1

3. Results and Discussion

3.1 Experiment on the effect of disinfectants on the survival and grafting compatibility of bud grafts of different pomelo varieties

Through the experiment, we obtained the following results: The results in Tables 1 and 2 show that disinfecting the shoots of various pomelo varieties with 10% Hypo calci for 10 minutes resulted in the lowest infection rate and the highest survival rate, significantly different from the other treatments, except for the Da xanh pomelo variety, which, when disinfected with 0.05% HgCl for 10 minutes, and the LCC pomelo shoots, which, when disinfected with 0.1% HgCl for 10 minutes, showed no difference compared to 10% Hypocanxi for 10 minutes.

This result is similar to the research findings of Trần Văn Đạt [16] amd Silva, 2019 [17] when using 10% hypocalci for 10 minutes to sterilize CCM buds for grafting, achieving the lowest infection rate.

Table 1: The effect of disinfectants and sterilization time on the infection rate of grafted pomelo varieties

	Variety (S)			
Disinfectant	dx	dlc	lcc	T-MEAN
hyp10	0.01(0.57)c	0.010(0.57)c	0.01(0.57)b	0.01
Hg0.1-10	22.22(23.69)b	44.44(41.75)b	11.11(12.13)b	25.92
Hg0.05-10	0.010(0.57)c	77.77(66.30)a	44.44(41.75)a	40.74
Hg0.1-5	22.22(23.69)b	44.44(41.75)b	44.44(41.75)a	37.03
Hg0.05-5	55.55(48.24)a	44.44(41.75)b	55.55(48.24)a	51.85
S-MEAN	20.00	42.22	31.11	31.11

Note: In the same column, characters that follow each other are not significantly different at the 5% level according to the DMRT test. The numbers in parentheses are the data converted to Arcsine (Sqr/100) before processing

Table 2: The effect of disinfectants and sterilization time on the survival rate of grafted pomelo varieties

	Variety (S)			
TREAT (T)	dx	dlc	lcc	T-MEAN
Hyp10	99.99(89.43)a	99.99(89.43)a	99.99(89.43)a	99.99
Hg0.1-10	77.78(66.30)b	55.56(48.25)b	88.88(77.86)a	74.07
Hg0.05-10	99.99(89.43)a	22.22(23.70)c	55.56(48.25)b	59.26
Hg0.1-5	77.78(66.30)b	55.56(48.25)b	55.56(48.25)b	62.96
Hg0.05-5	44.44(41.75)c	55.56(48.25)b	44.44(41.75)b	48.15
S-MEAN	80.00	57.78	68.89	68.89

Note: In the same column, characters that follow are not significantly different at the 5% level according to the DMRT test. The numbers in parentheses are the data converted through Arcsine (Sqr/100) before processing

Table 3. The effect of disinfectants and sterilization time on the grafting success rate of various grapefruit hybrids

	Variety (S)			
TREAT (T)	dx	dlc	lcc	T-MEAN
Hyp10	77.78(66.30)ab	77.78(66.30)a	55.56(48.25)ab	70.37
Hg0.1-10	55.56(48.25)bc	22.22(23.70)b	66.67(54.47)a	48.15
Hg0.05-10	99.99(89.43)a	22.22(23.70)b	33.33(35.26)ab	51.85
Hg0.1-5	55.56(48.25)bc	55.56(48.25)ab	22.22(23.70)b	44.45
Hg0.05-5	22.22(23.27)c	33.33(35.26)b	22.22(23.70)b	25.93
S-MEAN	62.22	42.22	40.00	48.15

Note: In the same column, characters that follow are not significantly different at the 5% level according to the DMRT test. The numbers in parentheses are the data converted through Arcsine (Sqr/100) before processing.

Table 4: The effect of rootstocks on grafting success and shoot growth

	Variety (G)			
Root graft (G)	Lcc	dlc	dx	V-MEAN
RLM	22.22(23.69)a	88.88(77.86)a	44.44(41.75)b	51.85
cm	0.01(0.57) b	33.33(35.26)b	55.55(48.24)ab	29.63
lcc	22.22(23.69) a	33.33(35.26)b	0.01(0.57)c	18.52
dx	0.01(0.57) b	88.88(77.86)a	77.77(66.30)a	55.55
G-MEAN	11.11	61.10	44.44	38.89

However, the rate of graft union after sterilization and grafting is a decisive factor for the success rate of the grafting combination [2, 11]. Therefore, the analysis results in Table 3 show that the grafting success rate of different pomelo varieties significantly differs when using different disinfectants for varying durations: For the Da xanh pomelo variety, disinfection with 0.05% HgCl or hypochlorite for 10 minutes resulted in the highest grafting success rates (100% and 77.78% respectively), significantly different from the other treatments. For the ĐLC grapefruit variety, disinfection with hypocanxi for 10 minutes and HgCl 0.1% for 5 minutes yielded significantly higher results compared to the other treatments (77.78% and 55.56%, respectively). As for LCC grapefruit, disinfecting the buds with 0.1% HgCl, 10% Hypocanxi, and 0.05% HgCl for 10 minutes yielded the best results (66.67%, 55.56%, and 33.33%) significantly different from the other two treatments.

Thus, the disinfectant with different concentrations and treatment times will determine the compatibility factor of the grapefruit grafting combinations. Through the experimental results, to enhance the success rate of grafting for each graft combination, we found that the following disinfectants with specific concentrations and treatment times should be used for each variety in order of priority to achieve the highest rate:

1. DX: HgCl 0.05%-5 minutes -> Hypo 10-10’-> HgCl0.1% 5-10’
2. DLC: Hipó -> Hg 0.1-5’
3. LCC: Hg 0.1-10’ -> Hypo 10-10’

3.2 Experiment on the influence of rootstocks on grafting success rate and shoot growth

The results in Table 4 show that different pomelo varieties have different grafting rates with rootstocks:

LCC has the best compatibility rate with RLM and itself (LCC) (22.22%), significantly different from the other two rootstocks, DX and CM.

Fig 1: LCC grafted on 2-month-old RLM rootstock

ĐLC has the best compatibility rate with RLM and DX (88.88%) but does not have good compatibility with CM and LCC.

Fig 2: Drought tolerance on DX rootstock at 30 days old

Fig 3: Drought tolerance on RLM rootstock at 30 days old

DX grafts well with itself and CM and RLM (77.77%, 55.55%, 44.4%). This confirms that it is necessary to accurately identify each type of rootstock suitable for each type of pomelo variety to ensure compatibility between the varieties, thereby achieving the highest grafting success rate. However, priority should be given to interspecies combinations (it is not advisable to graft on the same type of rootstock to avoid confusion with wild shoots, e.g., LCC grafted with LCC).

Fig 4: DX on CM graft rootstock 30 days old

Based on the above results, we recommend using rootstocks for grafting each type of scion as follows:
1. LCC: RLM
2. DLC: RLM or DX
3. DX: CM or RLM.

3.3 Experiment on the effect of growth regulators on the grafting success rate and development of various grapefruit graft combinations

The results shown in Table 5 indicate that BAP at a concentration of 1mg/l did not yield good grafting and growth rates for all graft combinations due to the high concentration of BAP affecting the development of the graft combinations. For the LCC grapefruit combination, the remaining BAP concentrations were not significantly different. For the ĐLC and Da xanh grapefruits, BAP did not play a role in the development of these graft combinations, as shown in Table 5 shows that in the environment without BAP supplementation, the graft combinations had the best grafting and growth rates, although they were not significantly different from the others. However, through sensory evaluation, we observed that with the addition of BAP, the apical buds of the ĐLC and DX grapefruit varieties developed better compared to the environment without BAP supplementation: The apical buds and leaves grew faster and better. Visually, one can notice the change in size of the apical buds compared to the apical buds of the grafted combinations grown in the MS environment without BAP.

Thus, BAP does not play a significant role in the grafting and development of the DLC, DX, and LCC grapefruit graft combinations.

Through the evaluation results of the growth of the grafted combinations of grapefruit varieties using growth regulators, we found that BAP (0-0.5mg/l) should be used in grafting to stimulate the grafted shoots to grow faster and better for all the tested grapefruit varieties.

Table 5: The effect of growth regulators on the grafting success rate and development of the grafted combinations of grapefruit varieties

BAP concentration (T) (mg/L)	lcc	dlc	dx	T-MEAN
0.0	22.22(16.16)a	88.88(95.58)a	44.44(44.35)a	51.85a
0.1	22.22(16.16)a	5.56(55.56)ab	44.44(44.35)a	40.74a
0.5	22.22(16.16)a	66.67(66.67)ab	33.34(25.29)a	40.74a
1.0	0.01(0.01)a	33.34(25.29)b	22.22(16.16)a	8.52b
V-MEAN	16.67	61.11	36.11	37.96

Note: In the same column, characters that follow are not significantly different at the 5% level according to the DMRT test. The numbers in parentheses are the data converted to Arcsine (Sqr/100) before processing

4. Conclusions

The research results show that factors such as disinfectants, rootstock types, and the concentration of the growth regulator BAP significantly affect the grafting success rate and the development of the grafted shoots of various pomelo varieties. Specifically:

The Green Skin Pomelo variety achieves a high grafting success rate when the buds are sterilized with 0.05% $HgCl_2$ for 5 minutes or 10% $Ca(ClO)_2$ for 10 minutes, grafted onto Rough Lemon or Sweet Orange rootstocks, and cultured in MS liquid medium supplemented with 0–0.5 mg/L BAP.

The Long Co Co grapefruit variety is suitable when the buds are treated with 10% $Ca(ClO)_2$ or 0.1% $HgCl_2$ for 10 minutes, grafted onto Rough Lemon or Sweet Orange rootstocks, and cultured in MS liquid medium supplemented with 0–0.5 mg/L BAP.

The Cam Leaf Sugar Grapefruit variety achieves good results when using $Ca(ClO)_2$ 10% or $HgCl_2$ 0.1% for 5–10 minutes to disinfect the buds, grafted onto Rough Lemon or Green Skin Grapefruit rootstocks, and cultured in MS liquid medium supplemented with 0–0.5 mg/L BAP.

It is necessary to continue researching the optimization of factors affecting the survival rate, grafting success, and growth of the shoot apex in tissue culture, in order to perfect the production process of disease-free citrus plants.

- Conduct a second grafting to perfect the seedlings and enhance their uniform growth potential.
- Conduct disease-free testing of the plants after grafting to ensure quality and biosafety before production.

5. References

1. Nguyễn Văn Hòa, Trần Thị Cẩm Tú. Kỹ thuật vi ghép trong nhân giống sạch bệnh cây có múi. Tạp chí Khoa học và Công nghệ Nông nghiệp Miền Nam. 2020; 7(2):65-72. [Vietnamese]
2. Navarro L, Juárez J, Pina JA. Micrografting techniques for the production of pathogen-free citrus plants: An overview. Scientia Horticulturae. 2020; 268:109380.
3. Lê Hồng Khánh. Ảnh hưởng của gốc ghép đến sinh trưởng và năng suất của cây cam Xoàn. Tạp chí Nông nghiệp và Phát triển nông thôn. 2021; 4(3):112-119. [Vietnamese]
4. Trần Thanh Long. Nghiên cứu ứng dụng vi ghép để sản xuất cây giống sạch bệnh cam sành tại ĐBSCL. Viện Cây ăn quả miền Nam, Báo cáo chuyên đề kỹ thuật, 2022. [Vietnamese]
5. Trần Quang Huy *et al*. Ứng dụng vi ghép trong phòng trừ bệnh greening trên cây cam quýt. Tạp chí Bảo vệ thực vật Việt Nam. 2022; 10(2):45-50. [Vietnamese]
6. Nguyễn Thị Minh Châu. Ứng dụng kỹ thuật vi ghép trong sản xuất cây giống sạch bệnh cây có múi. Tạp chí Nông nghiệp Công nghệ cao. 2019; 6(1):55-63. [Vietnamese]
7. Nguyễn Hữu Thắng. Vi ghép cây có múi: giải pháp bền vững trong nhân giống sạch bệnh. Báo Nông nghiệp và Phát triển nông thôn, 2020. [Vietnamese]
8. Lê Thị Hương Giang. Khả năng áp dụng vi ghép trong sản xuất đại trà cây giống cam Vinh. Tạp chí Khoa học – Đại học Vinh. 2021; 9(4):71-78. [Vietnamese]
9. Nguyễn Thị Mai Hương. Hiệu quả kinh tế của kỹ thuật vi ghép trong sản xuất giống bưởi da xanh sạch bệnh. Tạp chí Kinh tế Nông nghiệp và Phát triển Bền vững. 2023; 5(1):57-65. [Vietnamese]
10. Lương Văn Chính. Đánh giá hiệu quả sử dụng vi ghép trong bảo tồn nguồn gen cây bưởi cổ. Hội thảo Quốc gia về Công nghệ sinh học thực vật, 2021. [Vietnamese]
11. Nguyễn Văn Dũng. Ảnh hưởng của kỹ thuật vi ghép đến tỉ lệ sống và khả năng sinh trưởng của cây giống cam ghép. Tạp chí Khoa học Trường Đại học Cần Thơ. 2020; 56(1):20-27. [Vietnamese]
12. Nguyễn Thị Kim Chi. So sánh hiệu quả giữa phương pháp vi ghép và ghép truyền thống trong nhân giống cây có múi. Tạp chí Nông nghiệp Việt Nam. 2023; 3(5):88-94. [Vietnamese]
13. Đào Văn Đức, Nguyễn Hữu Lộc. Tối ưu hóa quy trình vi ghép cam sành trên gốc ghép kháng bệnh. Tạp chí Sinh học và Công nghệ Sinh học. 2021; 13(2):102-109. [Vietnamese]
14. Wang N, Trivedi P, Folimonova SY. Advances in Citrus Huanglongbing (HLB) research: Micrografting and early detection tools. Plant Pathology Journal. 2021; 37(4):299-308.
15. Phạm Quốc Hưng. Ứng dụng công nghệ nuôi cấy mô kết hợp vi ghép trong sản xuất cây giống sạch bệnh. Kỷ yếu Hội nghị Công nghệ Sinh học toàn quốc, 2022. [Vietnamese]
16. Trần Văn Đạt. Nghiên cứu phản ứng tương hợp ghép giữa các giống cam quýt và gốc ghép bản địa. Tạp chí Khoa học và Công nghệ – Bộ NN&PTNT. 2022; 17(3):34-41. [Vietnamese]
17. Da Silva JAT, Dobránszki J. Micropropagation and micrografting of citrus: Recent advances. Biologia Plantarum. 2019; 63(3):345-356.