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Micro-Grafting Technique to Produce Disease-Free Bac Son Yellow Tangerine (Citrus reticulata Blanco) and Muong Pon Orange (Citrus sinensis (L.) Osbeck) Trees

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1. Abstract

This research aims to determine the efficiency of the micro grafting approach for clonal multiplication of disease-free Bac Son yellow tangerine (*Citrus reticulata Blanco*) and Muong Pon orange (*Citrus sinensis (L.) Osbeck*) contributes to the economy and agriculture. It is still subjected to low yields resulting from genetic erosion, diseases, and environmental factors affecting it. The purpose of this research is to use the micro-grafting technique to propagate the disease-free stocks of the two varieties, namely the Bac Son yellow tangerine (*Citrus reticulata Blanco*) and Muong Pon orange (*Citrus sinensis (L.) Osbeck*). The technique involves growing seeds from the rootstock, namely Dien grapefruit, sour grapefruit and Chap seeds (*kaffir lime*), under aseptic conditions. Grafting was done using shoot tips of Bac Son yellow tangerine and Muong Pon orange on selected rootstocks. Parameters such as graft take, graft shoot height, and successful transplantation percentage were recorded after 20-30 days. It assesses the various times in sterilization, types of culture media and types of rootstock in propagation to arrive at the best way. Assessment of the variations involves using Statistical Analysis Software System SAS 9.1 in conjunction with Duncan's multiple range test. Research findings showed that MS medium gave a higher percentage of germination rates than grapefruit seeds and sour grapefruit and accept rootstocks. However, the germination percentage was low and proved better than N6 and B5 media. For the grafting success rate test, grapefruit rootstock achieved the highest rate of 73.33% (Bac Son yellow tangerine) and 66.66%, proving their compatibility. The study also determined that plants germinating from grapefruit rootstock had higher survival rates of 86.67% and a shoot

height of 14.33 cm. Such evidence underscores the significance of substrate properties and rootstocks in boosting the process of citrus propagation and overall productivity.

Keywords: Citrus propagation, Grafting, Micro grafting, Muong Pon Orange, Bac Son Yellow Tangerine

2. Introduction

The Bac Son yellow tangerine (*Citrus reticulata Blanco*) and Muong Pon orange (*Citrus sinensis (L.) Osbeck*) hold significant economic and agricultural value in Vietnam (N. Hasan et al., 2019). The variety is consumed locally, especially the Bac Son yellow tangerine from the Lạng Son province, with a unique sweet taste and friable aroma that makes the fruit very valuable (Tran Nguyen et al., 2019). The province has 1,400 hectares of area under peanuts, and annually, it yields 3,000–3,500 tons and brings more than 100 billion VND (Branca et al., 2017). Like the Muong Pon orange, popular in the 1970s, the sort was once widely planted; some farmers planted about 100 trees per hectare (Cochard et al., 2021). Orange is the fruit of the citrus tree scientifically known as *Citrus sinensis*, which is a cross between a pomelo and mandarin tree and initially grown in the southern part of China, northeast of India, and Myanmar (Palangasinghe et al., 2024). Oranges, delightful oranges, have been recorded in Chinese literature since 314 BCE, and in 1987, they became the most widely grown fruit tree in the world (Nilsson, 2021).

Productivity and fruit quality are threatened by genetic degradation, declining yield and disease susceptibility of citrus. Extensive farming practices and the absence of gene selection have caused the dilapidation of citrus varieties to a point where they cannot give high-quality fruit (Ngo et al., 2021). However, this genetic weakening makes citrus less profitable because fruit yields are lower, fruit sizes are smaller, and flavours are inconsistent (Giao, 2021). Trees are also becoming more vulnerable to severe diseases such as Greening (Huanglongbing) and Tristeza, which lead to yellowing leaves, fruit drop, and overall decline, eventually killing trees (Thakuria et al., 2023). Other pests, such as the citrus leafminers, the red mites, the fruit flies, and the stem borers, attack the trees and weaken the trees, resulting in increased vulnerability to attacks by diseases (Rao & George, 2018).

Oranges, specifically sweet oranges (*Citrus sinensis (L.) Osbeck*) and tangerines (*Citrus reticulata Blanco*), are among the most valuable fruit crops globally because of their high nutritive value,

economic importance and resistance to climate change (Volk et al., 2023). However, its production is hampered by diseases, including Greening and Tristeza, which harm the production and quality of oranges. These diseases are transmitted through grafting and insects or Tree species like Asian citrus psyllid (*Diaphorina citri*); efforts to control them are in vain (Stover et al., 2018). Citrus is a perennial crop that was once affected by this disease. The affected trees are rendered unproductive and are usually replaced. The techniques used in cloning or propagating plants are ineffective since the planting material will always be contaminated by diseases, causing reinformation and reducing or dwindling orchards (Le & Ha, 2017).

Micrografting is one of the most effective solutions for producing disease-free citrus plants. This method entails joining a small shoot containing plant tissues free from the disease to a vigorous rootstock plant with matching genetic makeup of the eradicated plant to form a new plant (Kanwar et al., 2019). However, (Sharma et al., 2021) study highlighted that Micro-grafting coupled with in vitro propagation results in the generation of many true-to-type, pathogen and pest-free Citrus saplings, forming the basis for renewed orchards enhanced yield. The purpose of this research is to use the micro-grafting technique to propagate the disease-free stocks of the two varieties, namely the Bac Son yellow tangerine (*Citrus reticulata Blanco*) and Muong Pon orange (*Citrus sinensis (L.) Osbeck*). The objectives include determining an efficient micro-grafting technique, raising disease-free saplings, assessing the efficiency of the different grafting methods, and ensuring regular production of healthy Bac Son yellow tangerine and Muong Pho orange trees to support continuous planting.

3. Literature Review

3.1. General Overview of Citrus Trees

Orange and tangerine (*Citrus sinensis (L.) Osbeck* and *Citrus reticulata Blanco*) are juicy fruits of the *Rutaceae* family that ranks among the fruits that are grown abundantly worldwide (Richa et al., 2023). Citrus trees are wide canopies, large-leafed and rooted trees with continuous branches and year-round foliage (Hussain et al., 2021). Citrus belongs to Kingdom Plantae, order Rutales, and there are around 160-162 species identified in Tanaka's classification (Inglese & Sortino, 2019). Their origin is attributed to Southern Asia, ranging from India to the Himalayas, China, Philippines, Malaysia, Southern Indonesia, and Australia. The global area of citrus plantations is

at 1,361,449 hectares and yields 12,473,165 tons annually, earning a foreign exchange of about 6 billion USD. In Vietnam, Citrus fruit is 75,600 hectares, and the production volume ranges from 736,100 tons per year (Ngo et al., 2021). Although, (Shorbagi et al., 2022) study discussed that oranges and tangerines such as Citrus sinensis and Citrus reticulata Blanco are among the common fruits known for nutritional benefits and versatility in various production sectors. They contain vitamins A, B1, B9 and C, fibre, trace minerals, and antioxidants effective for combating heart diseases, cancer and inflammation (Maugeri et al., 2019; Tiwari et al., 2024).

3.2. Challenges in Citrus Cultivation

Some of the most important citrus diseases and pests cause significant yield loss, fruit quality damage, and tree life span reduction. (Dala-Paula et al., 2019) Highlighted prevalent citrus diseases, including Greening disease and Tristeza, harm citrus crop production worldwide. The greening disease is disseminated through transplants and insects, including Asian citrus psyllids (*Diaphorina citri)* and African citrus psyllids (*Trioza entree*) (Ghosh et al., 2018*)*. (Seethapathy et al., 2022) noted that infected trees display chlorosis, growth malformations of fruits, low sucrose levels, and late-life cycle drops, causing a substantial loss in production. In addition, a study by (Sun et al., 2024) identified that Tristeza affects the citrus plants using the citrus tristeza virus, which is usually transmitted by aphids and through the infected plant parts. Pests, diseases, and other factors, such as unfavourable environmental conditions, also have impacted the decline in the production of citrus fruits (Donkersley et al., 2018). However, (Mondal et al., 2020) discussed that pests hamper tree development, including citrus leaf miners, red spiders, stem borers, and fruit flies that harm twigs and fruits. Another factor highlighted by (Tran Nguyen et al., 2020) study which impacts citrus production, is temperature changes that affect the quality and quantity of the produce rainfall and soil health. However, (Biswas et al., 2020; Sun et al., 2024) studies discussed that citrus plant germplasm needs to embrace disease-tolerant varieties, and innovative propagation techniques such as micro-grafting to generate clean, high-quality citrus plant materials for high-quality yield.

3.3. In Vitro Propagation Techniques

Meristem culture is one of the most efficient micropropagation methods to produce pathogen-free plants, especially citrus (Lai & Lai, 2019). This structure implies that meristems, which consist of

the shoot tips and growing points, are free from systemizing infection causative agents such as viruses, bacteria, and fungi. Meristem culture entails the growth of a small part of the shoot apex that ranges approximately less than one-tenth of a millimetre in size and culture in a nutrient medium under specific conditions (Samridha & Chandra, 2024). Most plant viruses penetrate the plants in a way that they do not affect the meristematic tissues; this technique is very efficient in eradicating viruses such as the Citrus Tristeza Virus (CTV) and Greening Disease (Chauhan et al., 2019). (Wang et al., 2022) discussed that micropropagation also has some advantages over the traditional techniques of propagating plants, including seeds or grafting. It facilitates the production of numerous plants whereby millions of disease-free seedlings are grown within 1-2 years compared to what would take years when the conventional methods are used (Rymbai, 2024). However, (Abdalla et al., 2022) elaborated that micropropagation makes it possible to multiply high-quality plants and reduce genetic variation, maintain precise growing standards and produce fruits of high quality in all the plants. In contrast with grafting or budding techniques, the pathogens, viruses and bacteria present within the plant can be easily eliminated in micropropagation (Lai & Lai, 2019).

4. Methodology

4.1. Materials and Equipment

4.1.1 Description of plant materials

The plant materials used were rootstock seeds, Dien pomelo seeds, sour pomelo seeds and Chap seeds, which were vigorously resistant to diseases that the scions would be grafted upon. Bac Son yellow tangerine branches with dormant buds and Muong Pon orange branches were selected for micro-grafting. These branches taken from the mother trees help to develop quality, true-to-type, and disease-free plants for the sustainable plantation of the affected orchards.

4.1.2 Chemicals used in the process

The study employs an MS medium, a standardised basal growth media containing the basic plant growth requirements. The MS medium is one of the most commonly known basal growth media for plant tissue culture and contains all the necessary nutrient requirements for growth (Phillips & Garda, 2019). It is widely used as the primary carbon source because it promotes the proliferation

of cells (Tripathi et al., 2021). PGR includes Naphthalene Acetic Acid (NAA), belonging to Auxin, which encourages root initiation and Benzylaminopurine (BAP), belonging to Cytokinin, which encourages shoot formation and bud in the plant, as discussed by (Hesami et al., 2018).

4.1.3 Laboratory equipment and experimental setup

Table 1: Research equipment and tools

Device	Tool
Analytical balance	Pank
Magnetic stirrer	Cutlery
PH meter	Alcohol lamp
Autoclave	Measuring cup, measuring cylinder
Drying cabinet	Triangle vase
Lighting system	Plate (pea, pepper)
Water Distiller	Elastic belt
Sterile culture box	Plastic bag

4.2. Research Design

4.2.1 Experimental framework for in vitro rootstock production.

The methods used in the experimental procedure for the in vitro rootstock production, the seeds of grapefruit, sour grapefruit, and pomelo obtained from ripe fruits were used. The seeds underwent washings, washing with clean water for ten minutes, washing with 0.01% soap solution for ten minutes, and washing with tap water 5 times. Samples were washed in sterile distilled water thrice, then sterilized with 70% alcohol for 30 seconds and then washed again 5 times. The seeds were then placed in a solution containing 0.1% $HgCl_2$ for a given time of 5, 10, 15 or 20 min and then followed by a wash in sterile distilled water 5 times as described by (Rodboot et al., 2024). Post-sterilization, the seeds were separated into MS, N6 or B5 culture media in a completely randomised manner and data were recorded from three repetitions with 20 samples under each treatment. These cultures were kept at 25°C ± 2°C temperature, 60 % RH ± 5% humidity and light intensity of 2000-2500lux (M. N. Hasan et al., 2019; Nilwanshi, 2018).

4.2.2 Sterilization techniques for disease-free culture.

Preparing the disease-free culture starts with correctly identifying the growing tips of Bac Son yellow tangerine and Muong Pon orange branches. These samples were washed with clean water for 10 minutes, followed by 0.01% soap solution wash for 10 minutes, then rinsed with running tap water five times, as highlighted in (Sarkar et al., 2022) research. It is wise to wash the samples with sterile distilled water before taking them into the culture room and placing them inside the cabinet. To perform the sample preparation, the samples were washed for 5 minutes three times with sterile distilled water and then sterilized by wiping with 70% alcohol for 30 seconds before being rewashed with sterile distilled water 5 times. The samples were again cleaned with 0.1% $HgCl_2$ for periods ranging from 5 to 20 minutes, depending on the formula of the experiment (Singh et al., 2019). The samples were then subjected to 5 washes with sterile distilled water, after which culture was done in an appropriate growth medium.

4.2.3 Micro-grafting procedures and growth conditions.

The sequence of the micrografting procedure involves using Dien grapefruit, sour grapefruit or Chap seeds as rootstocks, which were germinated on MS medium and allowed to grow up to two cotyledons. Concerning the micropropagation phase of the Bac Son yellow tangerine and Muong Pon orange, the growing tips of 2mm are achieved at the shoot multiplication phase, as highlighted in (Chilukamarri et al., 2021) study. A grafting knife is employed to make a sharp cut on the rootstock across the cotyledon with a distance of 0.5cm from the cutting edge, and the in-vitro growing tip is placed at the centre of the cut surface. This was followed by micrografting of the plants, which were kept for observation for 20 days. The observations included the grafting success rate, number of successful grafting days, and shoot height.

4.3. Data Collection and Analysis

4.3.1 Key Performance Indicators

The criteria used in assessing the effectiveness of in vitro culture and micro grafting were acclimatization rate, shoot formation and percentage of successful graft. (Sandoval-Contreras et al., 2017) The study discussed that survival was evaluated after 20-30 days of culture by counting the proportion of free of contamination and with standard mycelium samples and no wilting. The efficiency of shoot regeneration is evaluated by the number of regenerated shoots per cultured

sample throughout 30 days, the coefficient and rate of shoot regeneration and the quality of the shoots identified by the stem thickness and the colour of the leaves. When using grafting, it is common to take 20 days post-grafting to assess its success concerning the time taken to form a scar, the survival rate for shoots, and the growth peak relative to the grafting point. These indicators must be met to guarantee the success of sterilization, regeneration, and micro grafting in citrus propagation (de Carvalho et al., 2021).

Statistical analysis methods

The analysis of the experimental data was done using SAS 9.1 software, a popular, reliable tool in agricultural science. The data were accumulated and processed in Microsoft Excel 2010 in the initial analysis phase. The Duncan multiple range test at a P-value of less than 0.05 using SAS software was performed to establish which developmental formulas differed significantly. The Duncan multiple range test was conducted at a P-value of less than 0.05 using SAS software. Thus, the Duncan and Newman-Keuls tests are helpful for this study to perform a request for multiple treatment means and to define statistically significant distinctions in the sterilization efficiency, the regeneration rate and the success of the grafting (Labiadh et al., 2023). This method makes it easy to distinguish between statistically significant changes to help make accurate conclusions concerning the efficiency of sterilization, the regeneration rate, and the success of grafting in citrus propagation.

5. Results

5.1. Effectiveness of In Vitro Rootstock Production

The results represent the latter, which affects the infection, death, and cleaning rates of three rootstock seed samples (Dien Grapefruit, Sour grapefruit and Chap tree (*kaffir lime*), after 20 days of sterilization by HgCl2 0.1%). It thus analyzes the effect of sterilization duration on seed quality and viability. The rate of infection is considerably reduced with time. For example, for Dien Grapefruit, the infection rate in distilled water control (0 minutes) equals 5.00%, 6.67% for Chap Tree and Sour Grapefruit 8.33%. Sterilization for 20 minutes reduces the infection rate to 5.00% for Dien Grapefruit and sour grapefruit, 5.00% and 13.33% for Chap Tree, respectively, and the p-value is significant ($p < 0.05$) (Table 2). The length of sterilization of the seeds can

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determine the success of the rootstocks in carrying out the disease-free seeds and subsequently assess the infection and mortality rate in the study. Prolonging this process might decrease or eliminate contaminants but result in seed mortality rather than germination and growth.

As with longer sterilization time, the mortality rate increases after 15 to 20 minutes. The mortality rates at 20 minutes are 66.67 % for Dien Grapefruit, 71.67 % for Sour Grapefruit, and 71.67 % for Chap trees, presumably bad due to the survival of the seedling at higher sterilization times. This implies that the exposure to HgCl2 may be toxic to the seeds for a long time. The highest clean-survival rate is for the 10-minute sterilization time, Dien Grapefruit was 78.33 %, Sour Grapefruit 70.00 %, Chap tree 65.00 %. This implies that 10-minute sterilization strikes an ideal balance between seed viability control and infection. In this case, decreasing sterilization times to 15 and 20 minutes are associated with less clean surviving cells, while diminishing returns are observed with follow-up sterilization times of 10 minutes or less (Table 2). The study's outcome can be achieved by showing the viability of the rootstocks from the seeds obtained through varying durations of seed sterilization. As the duration of $HgCl_2$ exposure to the seed increases, the germination percentage is affected and depressed. The optimal 10-minute sterilization is the best way to destroy disease transmission and maintain high survival rates in micro-grafting and propagation of Bac Son yellow tangerine and Muong Pon orange trees.

Table 2: Effect of sterilization time on infection, mortality, and clean-survival rates of rootstock seed samples (after 20 days).

CT	Sterilization time of HgCl2 0.1% (minutes)	Infection rate (%)			Mortality rate (%)			Clean-survival rate (%)		
		Dien Grapefruit	Sour Grapefruit	Chap Tree	Dien Grapefruit	Sour Grapefruit	Chap Tree	Dien Grapefruit	Sour Grapefruit	Chap Tree
1(DC)	Distilled water aseptic	100 ^{a}	100 ^{a}	100 ^{a}	5,00^{d}	6,67^{d}	8,33^{d}	0,00^{d}	0,00^{d}	0,00^{d}
2	5	40,00^{b}	40,00^{b}	38,33^{b}	15,00^{c}	16,67^{c}	16,67^{c}	50,00^{b}	48,33^{b}	45,00^{b}

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3	10	13,33^{c}	16.67 ^{c}	21.67 ^{c}	15.00 ^{c}	16.67 ^{c}	20.00 ^{c}	78.33 ^{a}	70.00 ^{a}	65.00 ^{a}
4	15	8.33 ^{dc}	11.67 ^{c}	10.00 ^{d}	43.33 ^{b}	41.67 ^{b}	46.67 ^{b}	50.00 ^{b}	48.33 ^{b}	43.33 ^{b}
5	20	5.00 ^{d}	5.00 ^{d}	13.33 ^{d}	66.67 ^{a}	71.67 ^{a}	71.67 ^{a}	31.67 ^{c}	26.67 ^{c}	20.00 ^{c}
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
CV		10.06	7.9	12.07	12.49	10.31	7.9	10.08	6.25	11.78
LSD $_{0.05}$		6.32	5.16	8.33	6.77	5.95	4.86	7.97	4.55	7.69

5.2 Influence of substrate environment on the germination rate and growth of in vitro rootstock seed sample

The findings depicted the germination rate and average plant height of various rootstock seed samples: grapefruit, sour grapefruit, and accept seed exposed to different substrate environments, MS, N6 and B5, after 30 days of culture (Table 3). It has been ascertained that the type of substrate influences seed germination and early growth significantly. The germination rate depends on the type of substrate MS medium, which yielded the highest germination rate with all the rootstocks. In the case of the flower bud, the growth proliferation of grapefruit seeds was 88.33%, while for sour grapefruit, it was 81.67% and accept 75.00% in MS as compared to N6 and B5 where the growth rate was significantly low with P <0.05 (see Table 3). The N6 medium yielded germination rates of 76.67%, 68.33% for the grapefruit and accept variety, while the sour grapefruit gave 71.67%. Out of all the media used in the study, the B5 had the least germination, and their rootstocks were 71.67%, 63.33%, and 63.33%, respectively. There is a strong correlation between the choice of the MS medium and enhanced germination and seed growth in the selected rootstock study. This study shows that MS medium promotes higher germination and plant height and, consequently, conducive conditions to generate healthy citrus rootstocks that contribute to improved micro-grafting results.

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The average height of seedlings is not constant and depends on the media type. The average seedling height length from the experiment was as follows: grapefruit 8.03 cm, sour grapefruit 7.87 cm and accept 4.93 cm; the observation indicates that MS medium yielded higher seedlings by two times than a half-strength MS medium recorded below. The N6 shoot produced slightly less height than the control, at 7.57 cm, 7.47 cm and 5.03 cm, while the B5 generated the shortest at 7.23 cm, 7.17 cm and 4.63 cm. The findings show highly significant differences ($P < 0.05$) between the plots, showing that MS and N6 effectively support early growth compared to B5. On the other hand, for the accepted variety, the height differences were not generally found to be very meaningful since the (P value > 0.05) signifies that all the media grew alike (see Table 3).

Table 3: Effect of substrate environment on germination rate and growth of rootstock seed samples (after 30 days of culture).

CT TN	Environment	Germination rate (%)			Average height (cm)		
		Dien Grapefruit	Sour grapefruit	Chap Tree	Dien Grapefruit	Sour grapefruit	Chap Tree
1	MS	88.33a	81.67a	75.00 [a]	8.03 [a]	7.87a	4.93a
2	N6	76.67 [b]	68.33b	71.67a	7.57b	7.47b	5.03 [a]
3	B5	71.67 [c]	63.33 [b]	63.33 [b]	7.23 [c]	7.17 [c]	4.63 [a]
P		<0.05	<0.05	<0.05	<0.05	< 0.05	>0.05
CV%		2.11	4.69	4,12	0.69	3.19	ns
$LSD_{0.05}$		3.78	7.56	6.54	0.12	0.51	ns

Note: a, b, c… show significant differences at the confidence level $P < 0.05$ according to the Duncan comparison method

Table 4 shows that the number of new leaves and the diameter of the body of the rootstock seedlings after 30 days of culture depend on the substrate environment: MS, N6 and B5. The results suggest that the type of substrate the seeds are sown on significantly impacts early vegetative growth. The overall mean of the number of leaves also differs among the three media, where MS has the highest mean for all the rootstock types. The different Dien grapefruit seedlings cultivated in MS had 4.43 leaves, while the sour grapefruit averaged 4.33 leaves and Chap tree 4.13 leaves. The N6 medium gave slightly fewer numbers of leaves, specifically, 3.93 in Dien grapefruit, 4.00 in sour grapefruit, and 3.80 in Chap tree. B5 medium yielded a very low number of leaves; for

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instance, the third plates yielded 3.67, 3.63 and 3.27, respectively. The important variations ($P < 0.05$) imply that among the two media, the MS medium enhances the growth of the leaf the most, while the B5 medium is the least effective.

However, there is also variation in the average body diameter of the seedlings in the three media. In the MS medium, it has been found that Dien grapefruit, sour grapefruit, and Chap tree had body diameters of 1.92 mm, 1.91 mm and 1.58 mm. The N6 medium is slightly less than that, 1.87±0.15 mm in Dien grapefruit, 1.88±0.11 mm in sour grapefruit and 1.47±0.52 (see Table 4). The B5 medium's diameters were the smallest among the three categories, which were 1.84mm, 1.84mm, and 1.40mm, respectively. Therefore, the observable differences ($P < 0.05$) show that MS promotes a better thickness of stems than N6 and B5. These results conclude that the MS medium is optimal for seedling growth with relatively good development of leaves and stem thickness. The N6 medium ranks closely behind, while the B5 medium is the worst in promoting early rootstock development (Table 4).

Table 4: Effect of substrate environment on rootstock growth (after 30 days of culture)

CT TN	Environment	Average number of leaves (leaves)			Average body diameter (mm)		
		Dien Grapefruit	Sour grapefruit	Chap tree	Dien Grapefruit	Sour grapefruit	Chap tree
1	MS	4.43a	4.33a	4.13 [a]	1.92 [in]	1.91 [in]	1.58 [in]
2	N6	3.93 [b]	4.00 [b]	3.80 [b]	1.87 [b]	1.88 [b]	1.47 [b]
3	B5	3.67 [c]	3.63 [c]	3.27 [c]	1.84 [c]	1.84 [c]	1.40 [c]
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
CV%		1.66	0.84	1.09	0.3	0.6	0.9
$LSD_{0.05}$		0.15	0.08	0.09	0.01	0.03	0.03

Note: a, b, c… show significant differences at the confidence level $P < 0.05$ according to the Duncan comparison method

5.2. Micro-Grafting Success Rate

The findings indicate the impact of the rootstocks on the in vitro micrografting success rate of growing tips extracted from both Bac Son yellow tangerine and Muong Pon orange after 20 days (Table 5). On average, shoot height, successful grafting percentage, days taken for successful

transplanting, etc, are taken into account. The height of the developing shoot tips is not constant, but rootstock has some effect. For Bac Son yellow tangerine, the highest shoot tip growth is observed in the Dien grapefruit rootstock, having 0.32 cm; the least shoot tip growth was recorded in the Chap tree rootstock, with 0.21 cm, and the sour grapefruit rootstock recorded an increase of 0.26 cm. However, in Muong Pon orange, the Dien grapefruit rootstocks give the best shoot tips of height at 0.35 cm, and the sour grapefruit and Chap tree rootstocks at only 0.26 cm and 0.23 cm, respectively (see Table 5). The study substantiates that using rootstocks positively affects the outcome of micro-grafting in Bac Son growing yellow tangerine and Muong Pon orange. Consequently, rootstock Dien Grapefruit tended to have high shoot tip growth, proving that it is ideal for shoot elongation and grafting over Chap tree and sour grapefruit rootstocks.

Similarly, the selection of the rootstock also affects the grafting success. The comparative grafting success is highest in the grapefruit rootstock, namely Bac Son yellow tangerine at 73.33% and Muong Pon orange at 66.66 %. The findings revealed that sour grapefruit rootstock has relatively higher success rates of 56.67% and 60.00% and the lowest success rates have been reported by Chap tree rootstock, which is 40.00% and 43.33%, respectively (see Table 5). The results indicate a general significance of differences ($P < 0.05$), which points to the fact that Dien grapefruit enhances grafting efficiency. Thus, the time is less than or equal to 7 days for both Bac Son yellow tangerine and Muong Pon orange rootstock grapefruit. As for the rootstock, sour grapefruit takes slightly longer at 8 days and 8 days and 33 minutes for the Chap tree, 8 days and 33 minutes for Bac Son yellow tangerine, and nine days for Muong Pon orange.

Table 5: Effect of rootstock type on in vitro Micro grafting efficiency of growing tips of Bac Son yellow tangerine and Muong Pon orange (after 20 days of culture)

CT	Type of rootstock	Bac Son yellow Tangerine			Muong Pon Orange		
		Average height of DST (cm)	Successful grafting rate (%)	Number of days of successful TB transplantation (days)	Average height of DST (cm)	Successful grafting rate (%)	Number of days of successful TB transplantation (days)

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1	Dien Grapefruit	0.32 [a]	73.33 [a]	7.00	0.35 [a]	66.67 [a]	7.0
2	Sour grapefruit	0.26 [b]	56.67 [b]	8.00	0.26 [b]	60.00 [a]	8.33
3	Chap tree	0.21 [c]	40.00 [c]	8.67	0.23 [b]	43.33 [b]	9.00
P		<0.05	<0.05		<0.05	<0.05	
CV%		6.40	7.20		6.02	8.31	
$LSD_{0.05}$		0.04	9.25		0.03	9.41	

According to the Duncan method, the numbers a, b, c… represent significant differences at the confidence level P < 0.05.

5.3. Growth Performance of Micro-Grafted Plants

As shown in Table 6, using different types of rootstock affected the growth of micrografted Bac Son yellow tangerine trees (S0) in terms of survival rate, body diameter, body height, and the number of leaves. The study shows that rooting stock dramatically affects the growth rate of micro-grafted plants after transplantation. The grafting of trees has been carried out using the micrografting technique, with varying degrees of survival for trees from different rootstocks. The highest survival rate is thereby recorded in Dien grapefruit rootstock at 86.67% compared to Chap tree rootstock with 83.33% and sour grapefruit rootstock with the lowest survival rate of 70.00 % (Table 6). Therefore, the present data indicate that Dien grapefruit and Chap tree rootstocks have a more remarkable ability to support the micro-grafted Bac Son yellow tangerine trees rather than sour grapefruit.

Among the trees grafted on ten varieties, the trees grafted on Dien grapefruit had the most prominent stem diameter of 0.37 cm, sour grapefruit had stems of 0.33 cm in diameter, and Chap tree rootstock produced the minor stem diameter of 0.30 cm. The findings show a positive effect of Dien grapefruit rootstock on stem diameter because it improves stem growth, enhancing plant stability and access to nutrients. On the other hand, the body height of micrografted trees shows that Dien grapefruit rootstock has the tallest plant height of 14.00 cm, sour grapefruit plant is slightly shorter and has a height of 13.50 cm, whereas Chap tree rootstock has the shortest plant

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height of 11.33 cm. The number of leaves also differs; Dien grapefruit rootstock has the highest number, 4.67; sour grapefruit, 4.00; and Chap tree rootstock, 3.33 (Table 6).

Table 6: Effect of rootstock type on growth of Micro grafted Bac Son yellow tangerine tree (tree S0)

CT	Rootstock type	Survival rate (%)	Body diameter (cm)	Body height (cm)	Number of leaves
1	Dien Grapefruit	86.67a	0.37a	14.00 [a]	4.67a
2	Sour grapefruit	70.00 [b]	0.33b	13.50b	4.00b
3	Chap tree	83.33a	0.30 [c]	11.33 [c]	3.33 [c]
P		<0.05	<0.05	<0.05	<0.05
CV%		5.89	2.21	1.28	5.89
LSD0.05		9.41	0.01	0.33	0.47

According to the Duncan method, the numbers a, b, and c represent significant differences at the confidence level P < 0.05.

The findings illustrated the impact of different rootstock types on the growth characteristics of micro-grafted Muong Pon Orange tree growth, such as survival rate, body diameter, body height, and number of leaves (Table 7). As per the results, rootstock has a net effect on the growth and success of the micro-grafted trees. The survival rate of Muong Pon orange trees depends on the rootstocks. Among the rootstocks used, the highest survival rate is observed with Dien grapefruit root stock=80.00%, and the last one is with sour grapefruit rootstock at 63.33% and the Chap tree root stock in-between it at 76.67% (see Table 7).

A bigger stem diameter is observed from Dien grapefruit rootstock, measuring 0.38 cm, and sour grapefruit followed it with a measurement of 0.35 cm. In comparison, the most minor stem diameter is Chap tree rootstock measuring 0.32 cm. The study shows that Dien grapefruit rootstock enhances the stem's development, crucial for transporting nutrients and structural support. The growth performance in terms of body height differs only slightly, with Dien grapefruit rootstock

yielding the tallest plants with a mean height of 14.33cm, sour grapefruit yielding plants of 14.00 cm and Chap tree rootstock yielding the shortest plants with a mean height of 12.16cm. (see Table 7).

Table 7: Effect of rootstock type on the growth of micro-grafted Muong Pon orange trees (tree S0)

CT	Type of rootstock	Survival rate (%)	Body diameter (cm)	Body height (cm)	Number of leaves
1	Dien grapefruit	80.00 [a]	0.38a	14.33 [a]	5.33 [a]
2	Sour grapefruit	63.33 [b]	0.35 [b]	14.00 [a]	4.83 [b]
3	Chap tree	76.67 [a]	0.32 [c]	12.16 [b]	4.00 [c]
P		<0.05	<0.05	<0.05	<0.05
CV%		6.42	1.33	2.76	4.99
$LSD_{0.05}$		9.41	0.01	0.74	0.47

6. Discussion

The study aims to discuss an efficient micro grafting technique for rapid, disease-free propagation of Bac Son yellow tangerine and Muong Pon orange saplings. It evaluates various grafting methods, their success rates, and the effective production of healthy citrus trees. (Weerasinghe et al., 2024) The Study emphasised that micrografting contributes to orchard renewal, increases fruit yield, and cultivates pathogen-free citrus varieties. According to the findings, the sterilization time and substrate condition affect citrus rootstock seeds' germination, growth, and survival. However, (Acemi & Özen, 2019) report similar findings that optimising sterilization time allows for contamination while ensuring seed viability. A study (Daniels, 2024) highlighted that exposure to $HgCl_2$ for periods longer than 15 minutes increased mortality. Although not all the seeds died, this indicates potential toxicity for sterilisation-induced seed damage. (Moreira et al., 2019) Research indicated that while the level of contamination was effectively minimized through sterilization, seed germination capacity was also affected. The highest germination rates and seedling heights were obtained with MS medium rather than N6 and B5 media. However, (Sudheer et al., 2022) demonstrated that MS medium is optimal for in vitro plant growth and is beneficial from the

balanced macronutrient and micronutrient composition. (Cai et al., 2018) showed that MS medium also elevated seedling vigour by supporting nutrient uptake and metabolic activity, explaining the increase in growth in this study.

The results show that substrate environment and rootstock choice affected early vegetative growth and success of micro grafting in Bac Son yellow tangerine and Muong Pon orange. The results support that MS media are the best environment for rootstock growth as it exhibits the highest number of leaves and largest stem diameter compared to B5 and N6 media. (Sudheer et al., 2022) The Study elaborated that nitrogen concentration and other macroelements in MS medium enhance cell division and elongation, thus increasing plant germination progression and height. This aligns with (Chew et al., 2018) research that MS media is an optimal growth medium as it contains a balanced amount of nutrients. However, (Huang et al., 2022) elaborated that MS medium promotes nutrient uptake and metabolic activity, improving seedling vigour. Concerning substrate density, the study by (Shahzad et al., 2020) highlighted that using MS medium is much more effective, as it enhances metabolic rates and nutrient absorption. However, (Gaire et al., 2022) identified that vigorous rootstocks facilitate the uptake of nutrients, grease wounds, and ameliorate scion development. The findings by (Bowman et al., 2021)confirm that survival rates for better overall plant resilience are enhanced by choosing highly compatible rootstocks for Micro propagated citrus plants. Additionally, union formation was more successful within seven days for grapefruit rootstock than other rootstocks. These results point to the necessity of proper substrate optimization and rootstock selection in order to improve citrus propagation, increase graft success, growing plant vigor, and long-term sustainability of the orchard.

The findings of (the Guruchandran et al., 2024) study explored the capacity and efficiency of MS medium to provide the appropriate macronutrients, micronutrients and growth controllers that assist in seedling growth. Another study by (Haradzi et al., 2021) discussed that propagations on MS medium enhance root and shoot growth of citrus, thus enabling the development of robust plants with enhanced survival of the plantlets. Moreover, the study indicates that rootstock positively affects the in vitro micrografting outcome. The findings (Rasool et al., 2020) noted that sources with compatible and vigorous rootstocks enhance graft union formation and nutrient uptake, improving graft establishment. Furthermore, the rootstock sour graprfruit had a moderate

grafting effect. In contrast, the Chap tree rootstock had the lowest effect, that poor rootstocks lead to low marker compatibility and high graft failure rates. This is similar to the studies done by (Liu et al., 2023) that indicate that increased rootstocks help shorten the graft healing period and growth of scions.

The results also show that rootstock type significantly affects the growth performance of the Micro grafted Bac Son yellow tangerine and Muong Pon orange tree, especially in the survival rate, stem diameter, plant height, and leaf number. Dien Grapefruit rootstock had the highest overall survival rate in both citrus cultivars, except rootstock, which constituted the second best; sour grapefruit had the lowest survival rate. These findings are by (Faria-Silva et al., 2020) study that well-developed rootstocks increase the grafting success rate and vitality by increasing water and nutrient absorbance. Dien Grapefruit rootstock also greatly affected tree height and stem diameter, giving the thickest stem required to support the plant and transport nutrients to other parts of the plant. Curk et al. (2022) also noted that integrating rootstock increases stem diameter, improving plant performance. However, (Bitters, 2021) findings recorded for plant height whereby plants grown from Dien grapefruit rootstock had the highest average height, followed by sour grapefruit and Chap tree rootstocks. According to (Basile & DeJong, 2018), early vigour of the selected rootstocks influences the general improvement of tree size and canopy growth. Furthermore, the plants had the highest number of leaves in Dien grapefruit rootstock which supports its vigor for vegetation spread.

7. Conclusion and Recommendations

The outcome of this research reveals the effect of sterilization time, substrate environment, and rootstock kinds on the success and growth of Micro propagated, and Micro grafted Bac Son yellow tangerine and Muong Pon orange trees. Therefore, rootstock seeds underwent sterilization with $HgCl_2$ 0.1%, and the best measure of clean-survival rate with relatively low seed mortality was at 10 minutes. Substrate environment was also a critical factor, which showed that the germination rates, seedling height and vegetative growth were recorded as the highest on MS medium base, followed by N6 and the least on B5 base media. It also favoured better stem thickness and several leaves, which made it a better medium for early seedling emergence in MS. As for the micrograft success, the highest survival means, ground diameters and plant heights were observed in Dien

grapefruit rootstock over both Bac Son yellow tangerine and Muong Pon orange. The significance of the study is to point out that for enhanced efficiency in micro grafting and growth of citrus trees, correct sterilization time, quality of the substrate, and an appropriate choice in the rootstock are helpful.

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