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RESEARCH ARTICLE

Study of *in vitro* propagation and acclimatisation in *Polianthes tuberosa* cv. Pink Sapphire

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Abstract

Polianthes tuberosa cv. Pink Sapphire is one of the beautiful commercial flower varieties newly imported into Vietnam. However, detailed studies on the micropropagation and acclimatisation of this flower species to the local environment are lacking. To meet the production and commercial demand of this flower variety in Vietnams' An Giang province, the study was conducted to select optimal media for *in vitro* propagation and suitable environments for seedling acclimatisation under greenhouse conditions. Five experiments were encompassed in this study, consisting of sterilisation of initial explants, *in vitro* shoot regeneration from bulb scales, *in vitro* shoot multiplication, complete plantlet generation and acclimatisation of seedlings in the greenhouse. The results demonstrated that the most effective sterilisation method was using 0.2 % $HgCl_2$ for 15 min and then 10 % $Ca(OCl)_2$ for 10 min. Four-scaling bulbs cultured on MS media containing 2.5 mg/L BAP and 0.5 mg/L NAA were recommended for *in vitro* shoot regeneration. The best medium for *in vitro* shoot multiplication was MS medium supplemented with 2 mg/L BAP and 0.5 mg/L NAA. The suitable medium for complete plantlet regeneration was MS medium. To acclimatise Pink Sapphire in the greenhouse, the optimal substrate was a mixture of rice husk ash, coconut coir and sand in equal parts (1:1:1). The findings of this study provide a foundation for the rapid development of the *P. tuberosa* cv. Pink Sapphire variety in An Giang province of Vietnam.

Keywords: acclimatisation; *in vitro*; medium; Pink Sapphire; *Polianthes tuberosa*; propagation

Introduction

Tuberose (*Polianthes tuberosa* L.) belongs to the genus *Polianthes*, is native to Mexico and is popularly used as a fresh-cut flower in tropical and subtropical countries. The *P. tuberosa* cv. Pink Sapphire is a newly bred flower from a maternal plant (*P. tuberosa* × *P. howardii*) and a paternal plant (*P. tuberosa* 'Double') (1). It has beautiful double florets, pale pink and a nice fragrance. There are two main types of tuberose flowers. Genotypes of single-flowered plants are typically utilised for extracting essential oils, as well as for loose flowers and floral arrangements; in contrast, double types of tuberoses are employed for cut flowers and garden displays (2). *Polianthes tuberosa* cv. Pink Sapphire flower is not yet widely cultivated in Vietnam, as it is a newly introduced variety. *Polianthes tuberosa* cv. Pink Sapphire is not yet widely cultivated in Vietnam because it is a newly introduced variety. Currently, in An Giang province, the demand for this flower is high, but its supply is limited and prices are elevated due to the fact that the bulbs are primarily imported from Taiwan. Therefore, research on the mass propagation of Pink Sapphire bulbs is essential to ensure a timely supply of seedlings, reduce investment costs and satisfy the increasing demands of flower enthusiasts.

Compared to traditional propagation methods such as dividing tubers and cuttings, *in vitro* protocols greatly enhanced the propagation rate and allowed the production of disease-free seedlings in large quantities (3). The micropropagation effectiveness of plantlets is affected by various factors, such as genotype, explant kind, position of explants on the medium, different culture media, plant growth regulators and substrates for plantlet acclimatisation under greenhouse conditions (2–5). Tuberose is usually propagated through vegetative bulbs or bulblets instead of using seeds (3). In addition, the use of *in vitro* protocols involving different components of the whole plant for the generation of new tuberose plants is gaining popularity (6). Many studies on propagation by tissue culture have been published; however, there is limited information regarding the parameters that influence the *in vitro* efficiency of tuberose (2). This study was conducted to develop and optimise a process for the *in vitro* propagation and bulb acclimatisation of *P. tuberosa* cv. Pink Sapphire in Vietnams' An Giang province. This will facilitate the production of a large quantity of high-quality plantlets to satisfy the demands of flower growers.

Materials and Methods

Place and time

The experiments were conducted at Nguyen Nhu Experimental Garden and An Giang University, belonging to Vietnam National University Ho Chi Minh City in Long Xuyen Ward, An Giang Province, Vietnam, from April 2024 to March 2025.

Materials

Plant materials

The *P. tuberosa* cv. Pink Sapphire bulbs were collected from the Nguyen Nhu Experimental Garden (Long Xuyen Ward, An Giang Province, Vietnam), originally sourced from Taiwan. Bulbs with a diameter of 1.0–1.5 cm were selected for experiments.

Chemicals

The study utilised Murashige and Skoog (MS) medium as the nutrient medium in tissue culture and plant growth regulators (PGRs) such as α-naphthaleneacetic acid (NAA), 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ). These chemicals were purchased from Duchefa Biochemie B.V., Netherlands. Calcium hypochlorite or chlorine, $Ca(OCl)_2$ 70 %, was from Mindy Materials (India) and mercuric chloride, $HgCl_2$ 98 %, was from Xilong Chemical Co. Limited (China).

Methods

Experiment 1 - Sterilisation of initial Pink Sapphire explants

Pink Sapphire tuberosa bulbs were removed of all excess leaves and washed under running water for 20 min to remove soil and adhesive substances on the bulb surface. Next, in a sterile chamber, bulb samples were dipped into 70 % alcohol for about one min and then washed with sterile distilled water 3–4 times. After that, bulbs were treated with disinfectants ($Ca(OCl)_2$ and/or $HgCl_2$) according to the treatments designed specifically in Table 1. Finally, the samples were washed with sterile distilled water 4–5 times. After sterilising, the damaged parts were trimmed and then transplanted into the prepared MS medium. The experiment was conducted in a tissue culture room according to a randomised complete block design (RCBD) with six treatments (A_1–A_6), each treatment had four replications, each replication included 10 bulb samples and each bulb was cultured in a glass vessel. The culture samples were evaluated at one, two and three weeks after culturing with the following indicators:

$$\text{Infected sample rate (\%)} = \frac{F}{N} \times 100 \quad \text{(Eqn. 1)}$$

$$\text{Dead sample rate (\%)} = \frac{D}{N} \times 100 \quad \text{(Eqn. 2)}$$

$$\text{Surviving sample rate (\%)} = \frac{(N)-(D+F)}{N} \times 100 \quad \text{(Eqn. 3)}$$

Where, F- Number of infected samples; D- Number of dead samples; N- Total number of cultured samples

Experiment 2 - *In vitro* shoot regeneration from bulb scales

This experiment applied three kinds of explants, whole, double-scaling and four-scaling bulbs, to evaluate *in vitro* shoot regeneration ability (Fig. 1). These explants were cultured on MS media supplemented with BAP (0–2.5 mg/L) and NAA (0–0.5 mg/L) for 56 days (8 weeks). The experiment was carried out in an RCBD with nine treatments (B_1–B_9) shown in Table 2. Each treatment had four replications, with each replication consisting of two culture vessels and each sample was cultured in a separate vessel. Indicators were recorded at 2, 4, 6 and 8 weeks after culturing, including the number of regenerated shoots per explant, shoot height and number of leaves per explant.

Table 1. Treatments of sterilisation of Pink Sapphire tuberose explants

Treatment	$HgCl_2$		$Ca(OCl)_2$	
	Concentration (%)	Time (min)	Concentration (%)	Time (min)
A_1	0.2	15	10	10
A_2	0.2	20	-	-
A_3	0.1	20	-	-
A_4	0.1	15	10	10
A_5	-	-	10	20
A_6	-	-	10	25

$HgCl_2$: Mercury(II) chloride; $Ca(OCl)_2$: Calcium hypochlorite

Fig. 1. Three kinds of explants. A. Whole, B. Double-scaling, C. Four-scaling bulbs used in *in vitro* shoot regeneration.

Table 2. Treatments of explant kinds and PGRs in *in vitro* shoot regeneration

Treatment	Different explants utilised			Plant growth regulators (mg/L)	
	Whole bulb	Twin-scaling bulb	Four-scaling bulb	BAP	NAA
B_1	✓	-	-	0	0
B_2	✓	-	-	2.5	0
B_3	✓	-	-	2.5	0.5
B_4	-	✓	-	0	0
B_5	-	✓	-	2.5	0
B_6	-	✓	-	2.5	0.5
B_7	-	-	✓	0	0
B_8	-	-	✓	2.5	0
B_9	-	-	✓	2.5	0.5

PGR: Plant growth regulator; BAP: 6-Benzylaminopurine; NAA: Naphthaleneacetic acid

Experiment 3 - *In vitro* shoot multiplication

Explants collected from healthy shoots obtained from the experiment of *in vitro* shoot regeneration from bulb scales were cut off their leaves. Stem sections of Pink Sapphire tuberose, approximately 1.5 cm in length, were prepared and transplanted into MS shoot multiplication medium supplemented with 0.5 mg/L NAA, combined with one of two cytokinins, BAP or TDZ, at varying concentrations. The experiment was carried out in an RCBD with six treatments (C_1–C_6) shown in Table 3. Each treatment had four replications, with each replication consisting of two culture vessels and each sample was cultured in a separate vessel. Indicators were recorded at 2, 4 and 6 weeks after culturing, including the number of shoots, shoot height and number of leaves per explant.

Table 3. Different PGR treatments for *in vitro* shoot multiplication

Treatment	Plant growth regulators (mg/L)		
	BAP	TDZ	NAA
C_1	4	-	0.5
C_2	3	-	0.5
C_3	2	-	0.5
C_4	-	1.5	0.5
C_5	-	1.0	0.5
C_6	-	0.5	0.5

Notes: PGR: Plant growth regulator; BAP: 6-Benzylaminopurine; NAA: Naphthaleneacetic acid; TDZ: Thidiazuron

Experiment 4 - Complete plantlet generation

The strong shoots from the experiment of *in vitro* shoot multiplication were collected for the rooting experiment. Shoot samples were placed in different rooting media of MS and NAA. The experiment was conducted in an RCBD with five treatments (D_1–D_5) described in Table 4. Each treatment had four replications, with each replication consisting of two culture vessels and each sample was cultured in a separate vessel. Indicators were recorded at 1, 2, 4 and 6 weeks after culturing, including the number of roots, root length, shoot height and number of leaves per explant.

Table 4. Treatments of MS media and NAA in complete plantlet generation

Treatment	Nutrient media	NAA (mg/L)
D_1	MS	0
D_2	½MS	0
D_3	¼MS	0
D_4	½MS	1
D_5	¼MS	1

MS: Murashige and Skoog medium; ½MS: MS medium reduced mineral content by half; ¼MS: MS medium reduced mineral content by three-quarters; NAA: Naphthaleneacetic acid

Experiment 5 - Acclimatisation of Pink Sapphire in the greenhouse

Completely generated plantlets approximately 6 cm in height from experiment 4 were pruned to retain three leaves near the apex and transferred to grow in greenhouse conditions. They were grown in the C5 plastic pot (10.5 × 11.0 × 7.5 cm) containing sand, soil, coconut coir and rice husk ash. The experiment was conducted in an RCBD with five treatments (E_1–E_5) described in Table 5. Each treatment had four replications, with each replication consisting of two plants and each plant was grown in a separate pot. Fertiliser was applied every two weeks with the formula-Dau Trau HCMK organic mineral fertiliser (6 g/plant) + NPK chemical fertiliser (1.8 g/plant) (7). Water was sprayed in mist once a day in the morning. The temperature inside the greenhouse was maintained at 24–32 °C. Observation criteria were recorded four weeks after transplanting, consisting of the survival plant rate (%); plant height (cm); number of leaves/plant; and stem diameter (mm).

$$\text{Survival plant rate (\%)} = \frac{\text{number of survival plants}}{\text{total number of plants}} \times 100 \quad \text{(Eqn. 4)}$$

Table 5. Treatments of substrate components for acclimatisation of *Polianthes tuberosa* cv. Pink Sapphire in the greenhouse

Treatment	Ratio of substrate components (x:x:x)		
	Rice husk ash	Coconut coir	Sand
E_1	1	-	-
E_2	1	1	-
E_3	1	-	1
E_4	-	1	1
E_5	1	1	1

Data analysis

Data processing was performed using Microsoft Excel. Analysis of variance (ANOVA) and pairwise mean comparison of treatments by Tukeys' honest significant difference (HSD) test were then conducted using SAS 9.1 software.

Results and Discussion

Sterilisation of initial Pink Sapphire explants

Different concentrations of disinfectants and sample handling times affected the number of infected, dead and surviving bulbs at one, two and three weeks of *in vitro* culture, resulting in differences in the rates of these outcomes at the 5 % statistical significance level (Table 6). The A_1 treatment, which sterilised the Pink Sapphire bulbs using $HgCl_2$ 0.2 % for 15 min, combined with $Ca(OCl)_2$ 10 % for 10 min, gave the highest rate of surviving bulbs. Bulbs survived completely (100 %), nearly one-third (65.0 %) and more than half (57.5 %) after 1 week, 2 weeks and 3 weeks of culture, respectively.

Table 6. Rate (%) of the infected, dead and surviving samples at one, two and three weeks after culturing

Treatment	One week after culturing			Two weeks after culturing			Three weeks after culturing		
	Infected	Dead	Surviving	Infected	Dead	Surviving	Infected	Dead	Surviving
A_1	0^d	0^c	100.0^a	15.0^d	20.0^a	65.0^a	20.0^d	22.5^a	57.5^a
A_2	27.5^c	5.0^b	67.5^b	37.5^c	10.0^b	52.5^b	45.0^c	12.5^c	42.5^b
A_3	42.5^b	10.0^a	47.5^c	67.5^b	10.0^b	22.5^c	77.5^b	10.0^c	12.5^c
A_4	25.0^c	10.0^a	65.0^b	37.5^c	17.5^a	45.0^b	47.5^c	17.5^b	35.0^b
A_5	85.0^a	0^c	15.0^d	92.5^a	0^c	7.5^d	100.0^a	0^b	0^d
A_6	77.5^a	0^c	22.5^d	87.5^a	0^c	12.5^d	100.0^a	0^b	0^d
P-value	*	*	*	*	*	*	*	*	*
CV (%)	9.67	12.82	8.75	8.67	6.12	7.49	7.58	8.87	8.80

Notes: A_1–A_6: Treatments of disinfectants for sterilisation of Pink Sapphire tuberose; DAC: Days after culturing; CV: Coefficient of variation; Within the same group of average values, means followed by the same letter were not significantly different from each other; *: Significance at the 5 % level.

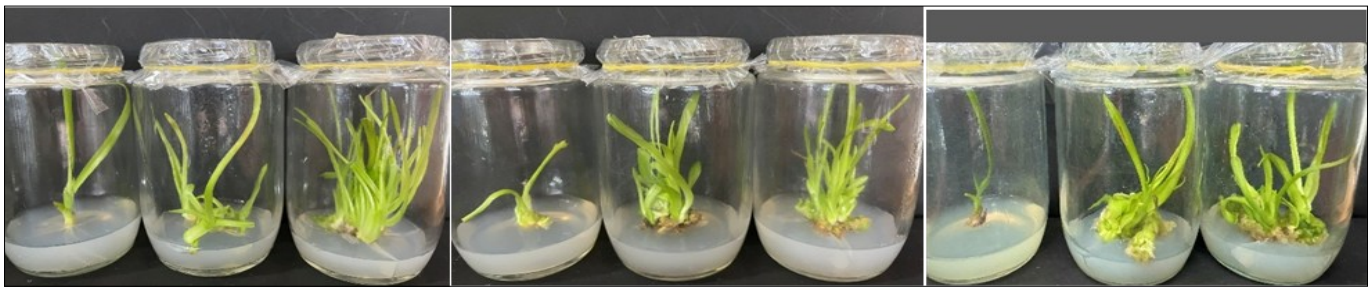
A previous study investigated the sterilisation stage of *in vitro* propagation in the mulberry plant and concluded that shoot tips and auxiliary buds sterilised with mercuric chloride 0.2 % for 10 min were the best to get sterilised and survive explants (8). While another study showed that the combined disinfectant, 70 % ethanol (20 sec) and $Ca(OCl)_2$ 10 % (15 min), was proposed as the best surface sterilisation protocol as it gave 100 % survival in explants of *Solanecio biafrae* (9). A study of *in vitro* culture in tuberose (*P. tuberosa* L.) found that treatments of single sterilant either by $Ca(OCl)_2$ or $HgCl_2$ were not effective for the percentage of survival (10). The combination of chlorox (sodium/calcium hypochlorite) and mercuric chloride helps to reduce bacterial contamination in *in vitro* culture of lily and tuberose species (11, 12).

In vitro shoot regeneration from bulb scales

The results of this experiment showed that PGRs such as BAP and NAA had a significant effect on shoot regeneration, e.g., the number of regenerated shoots, shoot height and number of leaves, in *P. tuberosa* cv. Pink Sapphire (Fig. 2, 3). PGRs like cytokinin and auxin are introduced to stimulate the initiation of shoot and root growth (13). BAP, one of the cytokinins, typically facilitates shoot growth and is applied in initiating shoot growth within plant culture. Meanwhile, auxins, e.g., NAA, are utilised to promote the development of roots (14, 15). In this study, the number of regenerated shoots in treatments with PGRs tended to be more than in treatments without PGRs. After eight weeks of culture, the treatments supplemented with BAP and NAA in all three bulb scales (whole, twin and four-scaling bulbs) had the highest number of shoots, followed by the treatments supplemented with only BAP and the lowest number in the treatments without growth regulators. The B_9 treatment, four-scaling bulbs cultured on MS media supplemented with 2.5 mg/L BAP and 0.5 mg/L NAA, had the highest shoot regeneration, reaching 9, 9, 10 and 14 shoots/bulb after two, four, six and eight weeks of culture, respectively (Fig. 3A). Shoot height in treatments without PGRs was often higher than in treatments with PGRs and treatments B_1 and B_7 had the tallest shoots (Fig. 3B). A previous finding demonstrated that the MS medium without NAA and BAP was best for the formation of roots and shoots and the addition of BAP and NAA in the medium decreased shootheight in tissue culture of potato and basil species (16, 17). However, the mechanism of these results has not been studied. After eight weeks of culture, the number of leaves in treatment B_3 was the most, followed by treatments B_8 and B_9 (Fig. 3C). Therefore, the treatment of four-scaling bulbs on MS media with 2.5 mg/L BAP and 0.5 mg/L NAA was proposed as the best treatment for *in vitro* shoot regeneration in *P. tuberosa* cv. Pink Sapphire.

In vitro shoot multiplication

Through 6 weeks of *in vitro* culture, shoot multiplication showed significant differences among six treatments (C_1–C_6) of PGRs (Fig. 4). The number of shoots and shoot height started to increase from the 2nd week and gradually rose until the 6thweek, while the number of leaves began later, forming from the 4th week onwards (Fig. 5). During the culture weeks, the C_3 treatment stimulated the largest number of shoots to arise and had a statistically significant difference compared to the other treatments (Fig. 5A). Treatment C_5 gave the best shoot height (7.66 cm, 10.56 cm and 12.40 cm at the 2nd, 4th and 6th week, respectively). Shoot height in treatment C_3 was lower than in treatments C5, C_4 and C_1 (Fig. 5B); however, shoots in treatment C_3 were stronger and more sturdy. Leaves began to appear from the 2nd week of culture. In the 2nd week, leaves only appeared in the C_3 and C_1 treatments. In the following weeks, the number of leaves in the C_3 treatment developed rapidly and was significantly larger than in the other treatments (Fig. 5C). Therefore, the C_3 treatment (MS medium supplemented with 2 mg/L BAP and 0.5 mg/L NAA) was the optimal treatment for *in vitro* shoot multiplication in Pink Sapphire tuberose. The previous study of *in vitro* propagation of *P. tuberosa* flowers demonstrated that MS medium supplemented with BAP and NAA increased multiple shoot rates (18). Another finding found that MS medium supplemented with 0.5 mg/L NAA combined with 1.0–2.0 mg/L BAP gave the highest number of shoots in *Stevia rebaudiana* (19).



B_1 B_2 B_3 B_4 B_5 B_6 B_7 B_8 B_9

Fig. 2. The differences in explant growth at the 8thweek in bulb kinds and PGRs in *in vitro* shoot regeneration. B_1–B_9: Treatments of explant kinds and PGRs.

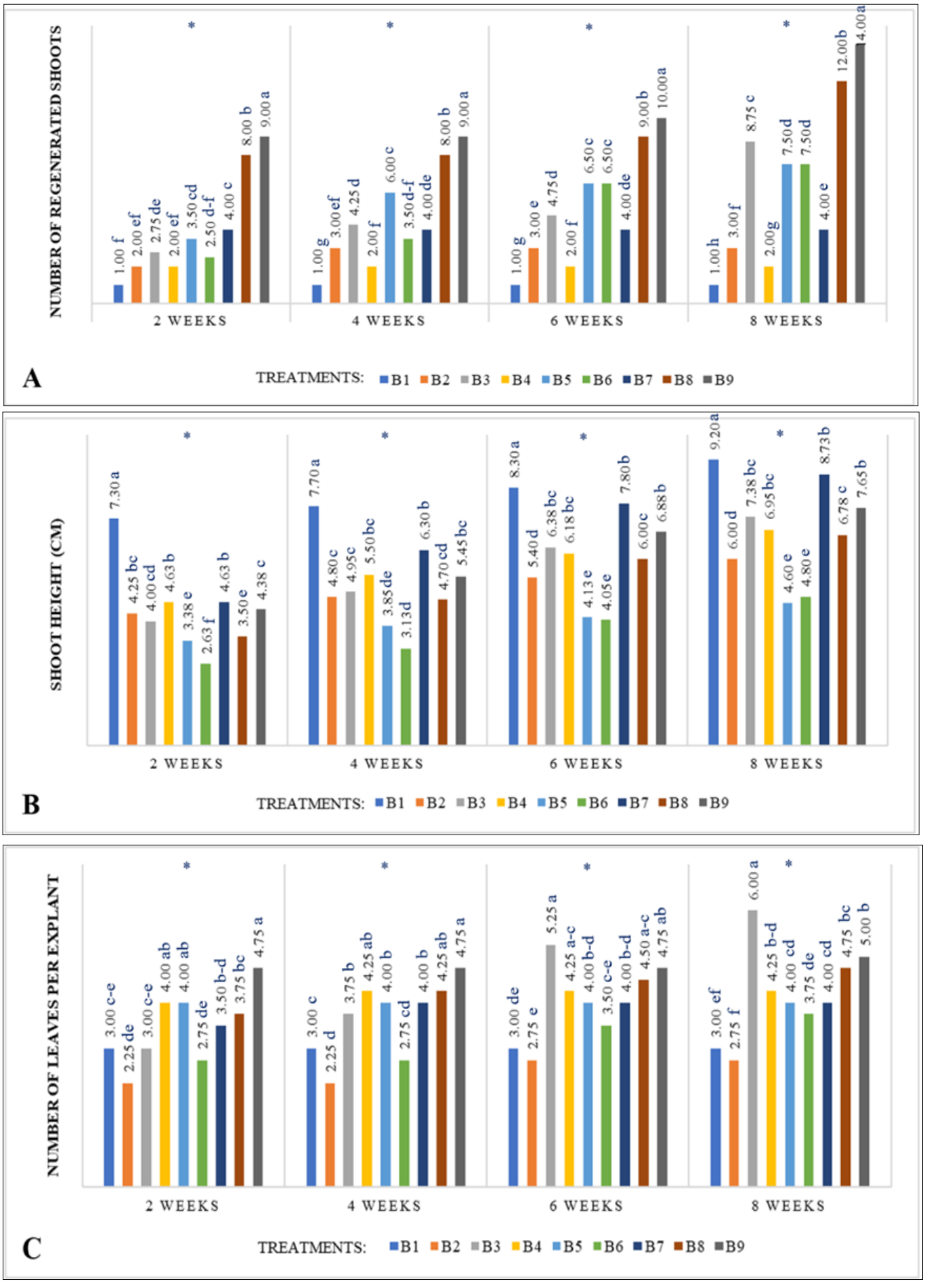


Fig. 3. Bulb scales in the different treatments of *in vitro* shoot regeneration. A. Number of regenerated shoots, B. Shoot height, C. Number of leaves per explant. B_1–B_9: Treatments of explant kinds and PGRs.

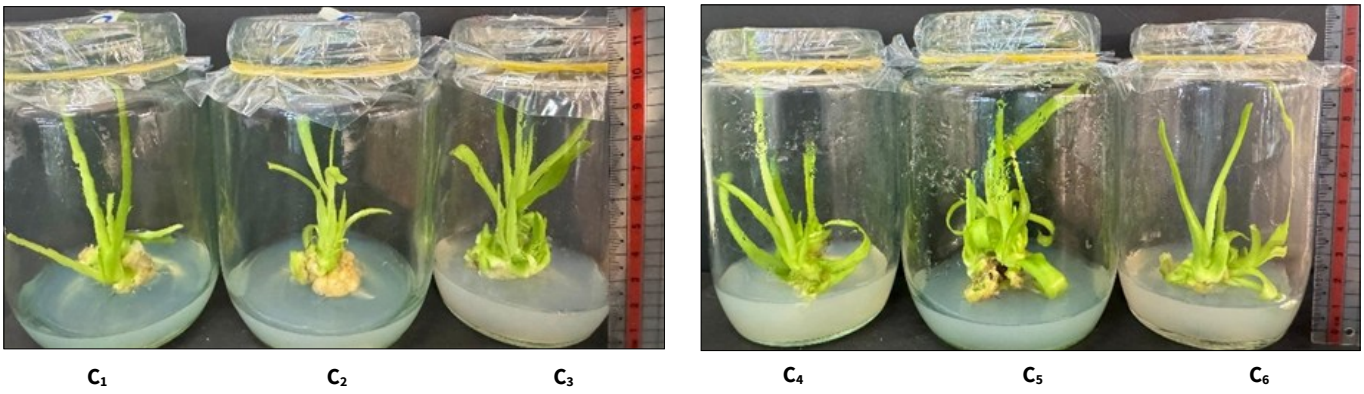


Fig. 4. The differences in shoot growth at the 6th week in six treatments of PGRs. C_1–C_6: Treatments of PGRs in *in vitro* shoot multiplication.

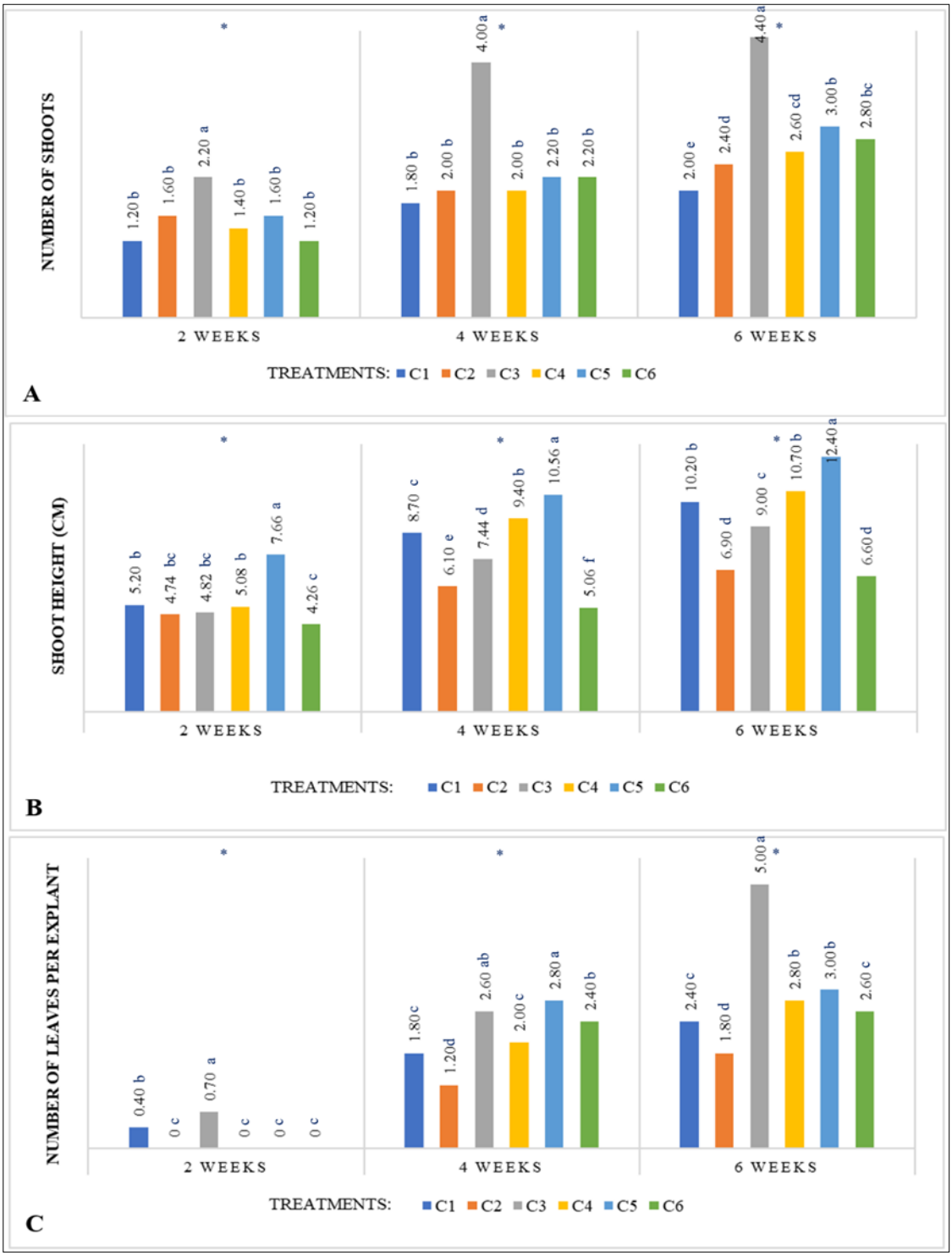


Fig. 5. A. Number of shoots, B. shoot height, C. Number of leaves of explants in the different treatments of *in vitro* shoot multiplication. C_1–C_6: Treatments of PGRs in *in vitro* shoot multiplication.

Complete plantlet generation

Over the weeks of culture, there were statistical differences among the environmental treatments in the formation of the number of roots and leaves and the growth in root length and shoot height (Fig. 6, 7). The highest number of roots and leaves was recorded in treatments D_1 and D_2 and there was no statistical difference between these two treatments in most weeks of culture, especially in the 4th and 6th weeks (Fig. 7A, B). Root length and shoot height in treatment D_1 were the highest and significantly different from those in other treatments (Fig. 7C, D). Thus, treatment D_1, MS medium without NAA, was the optimal medium for complete plantlet regeneration, followed by D_2, ½MS medium without NAA. This also showed that NAA was not effective in complete plantlet generation. Previous tissue culture studies also suggested that MS medium was more suitable for the growth of *in vitro* plantlets than other MS media strengths, such as ½MS and ¼MS (20–22). Furthermore, previous findings reported that plantlet growth, especially in shoot height, in *in vitro* culture was notably influenced by the MS media without growth regulators (13, 23).

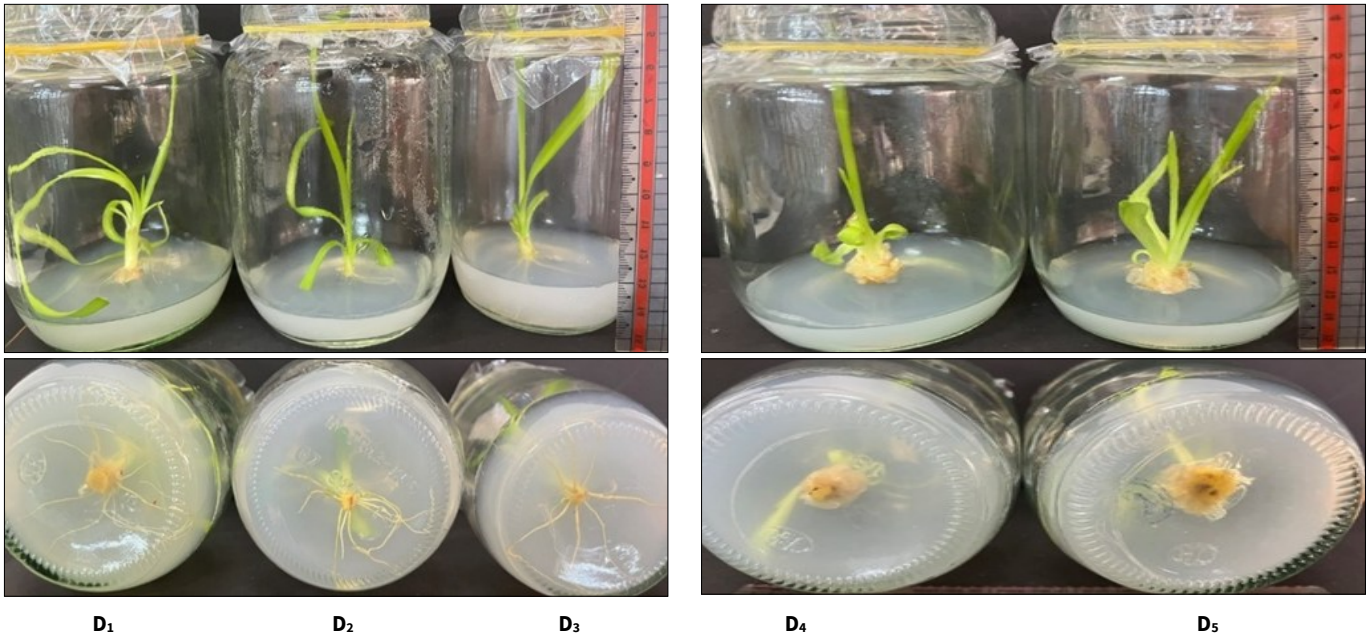
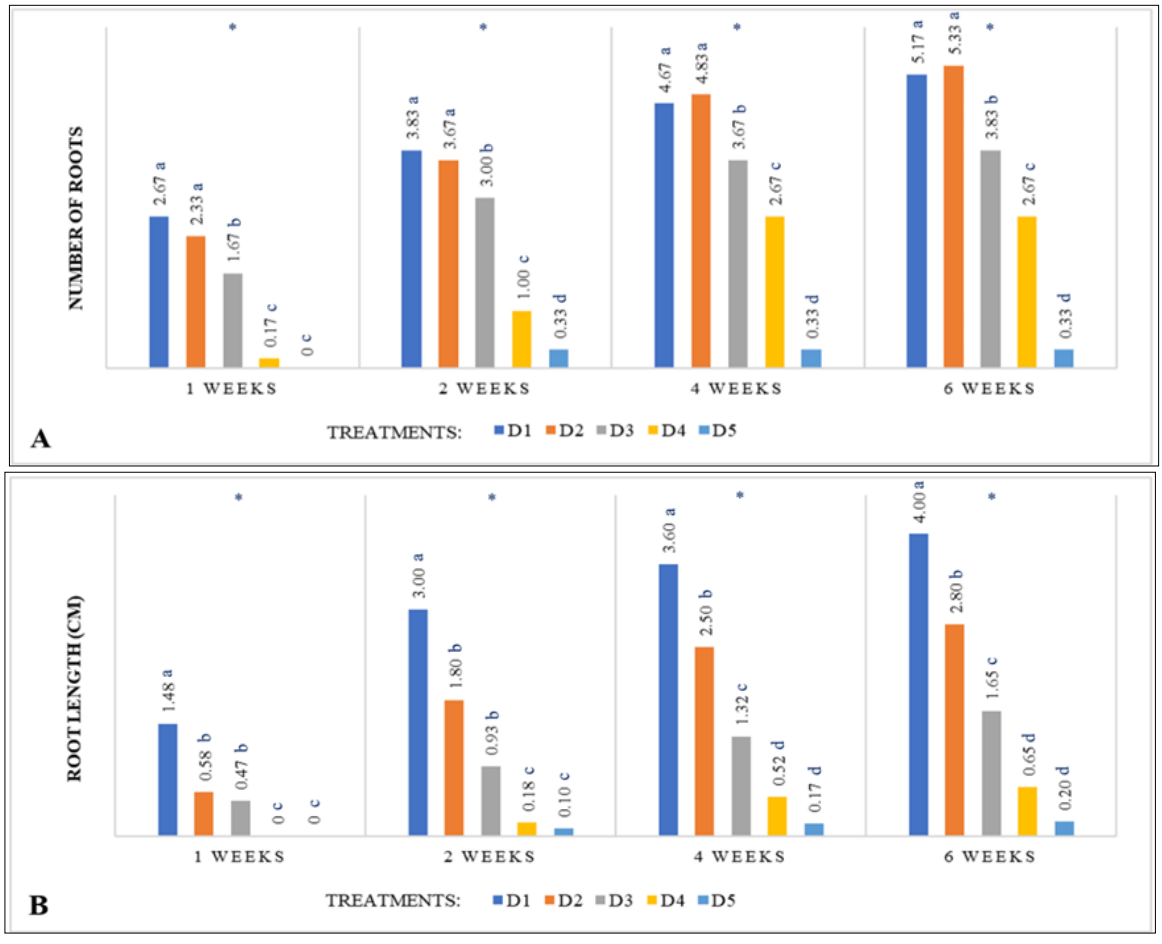


Fig. 6. The differences in shoot and root growth at the 6th week in treatments of complete plantlet generation. D_1–D_5: Treatments of complete plantlet generation.



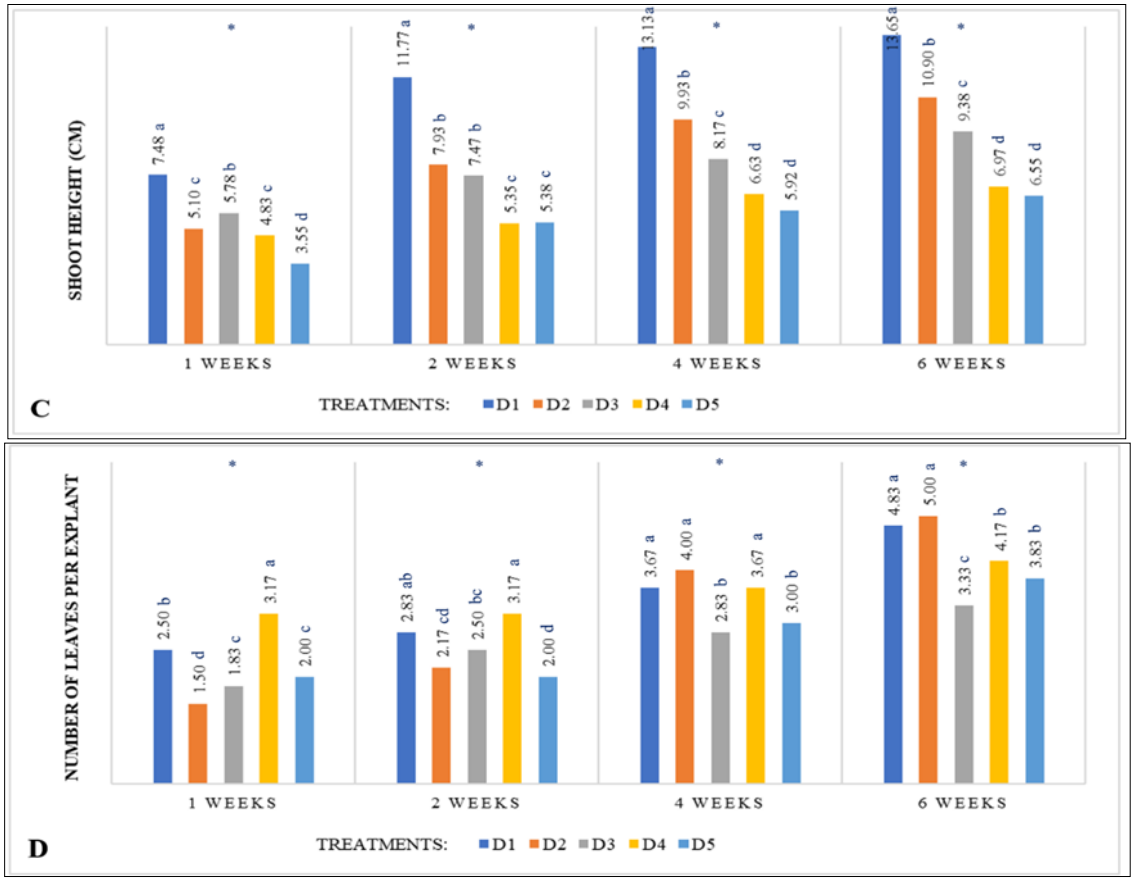


Fig. 7. A. Number of roots, B. Root length, C. Shoot height, D. Number of leaves of explants in the different treatments of complete plantlet generation. D_1–D_5: Treatments of complete plantlet generation.

Acclimatisation of *Polianthes tuberosa* cv. Pink Sapphire in the greenhouse

Healthy Pink Sapphire seedlings were grown in different substrates under greenhouse conditions. The results showed that seedling survival and growth among treatments were statistically significant at the 5 % level (Table 7, Fig. 8). Plants in treatments E_3 and E_5 were recorded as surviving completely for 4 weeks after transplanting; meanwhile, the survival of seedlings in other treatments only reached 62.5–87.5 %. Plant height in treatment E_5 was highest, followed by treatments E_3 and E_4 and the height in these treatments was significantly higher than in the other treatments. The number of leaves formed among the treatments exhibited minor differences, ranging from 6.38 to 7.75 leaves/plant and treatment E_5 had the highest number of leaves/plant. There were differences in stem diameter between the substrate treatments. The plants in treatment E_5 had the largest stem diameter, followed by E_3 and the diameter in these two treatments was significantly higher than in the other treatments. Therefore, treatment E_5, which consists of rice husk ash, coconut coir and sand in a 1:1:1 ratio, is the most optimal substrate for the survival and growth of Pink Sapphire seedlings under greenhouse conditions. Previous studies pointed out that the combined substrate of rice husk ash and coconut fibre is suitable for the survival and growth of *in vitro* seedlings of *Anthurium scherzerianum* and *Oncidium baueri* in greenhouse conditions (24, 25).

Conclusion

The study proposed the optimal media for *in vitro* propagation and the best substrate for acclimatisation in *P. tuberosa* cv. Pink Sapphire in the condition of An Giang province, Vietnam. The combination of $HgCl_2$ and $Ca(OCl)_2$ disinfectants helps achieve the highest sterilisation efficiency for the culture explants and the concentration ($HgCl_2$ 0.2 % for 15 min combined with $Ca(OCl)_2$ 10 % for 10 min) resulted in the best survival rate for Pink Sapphire bulbs. PGRs enhanced shoot regeneration about the quantity of shoots and leaves, but limited the shoot height. In *in vitro* shoot regeneration, MS media with 2.5 mg/L BAP and 0.5 mg/L NAA assisted four-scaling bulbs to reach the best number of shoots per explant. MS medium

Table 7. Survival and growth of Pink Sapphire seedlings in treatments of substrate components in the greenhouse

Treatment	Survival plant rate (%)	Plant height (cm)	Number of leaves/plant	Stem diameter (mm)
E_1	62.5^d	11.88^b	7.13^a	5.63^b
E_2	87.5^b	9.13^c	6.38^b	4.75^c
E_3	100.0^a	14.13^a	7.38^a	6.19^a
E_4	75.0^c	13.89^a	7.25^a	5.69^b
E_5	100.0^a	15.33^a	7.75^a	6.31^a
P-value	*	**	*	*
CV (%)	5.15	8.64	6.64	5.34

Notes: E_1-E_5: Treatments of substrate components; CV: Coefficient of variation; Within the same group of average values, means followed by the same letter were not significantly different from each other; *: Significance at the 5 % level; **: Significance at the 1 % level.

Fig. 8. Growth of seedlings in different substrate treatments under greenhouse conditions after 4th weeks after transplanting: A. Samples of five treatments were collected to evaluate, B. Plants in five treatments in the greenhouse.

combined with 2 mg/L BAP and 0.5 mg/L NAA was the optimal medium for *in vitro* shoot multiplication. The suitable medium for complete plantlet regeneration is MS medium without NAA. The most optimal substrate for acclimatisation of Pink Sapphire in the greenhouse is a mixture of rice husk ash, coconut coir and sand in a 1:1:1 ratio. These findings contribute to improving the *in vitro* culture process of *P. tuberosa* cv. Pink Sapphire. This will enable its application in the mass production of this species, resulting in higher yields and reduced costs.

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Authors' contributions

NTMD conceived of the study and participated in its design and coordination. TTTH and DNTH carried out the experimental work in the laboratory and greenhouse. BPT participated in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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