Effect of culture medium, growth regulators, and organic additives on shoot regeneration from hybrid-seedsof Phalaenopsis

ASTRACT

Aim: The Phalaenopsis Orchid, also known as the Moth Orchid, is a magnificent multicolored big orchid that has become a popular indoor plant and is easily recognized nowadays. The study of identifying the effects of culture medium, growth regulators, and organic additives on shoot growth from regenerated-embryos of Phalaenopsis is requirement in the first three months.

Methodology: Shoots from hybrid-seeds of Phlaenopsis germination after 2 months used as materials.Experiments were designs with the effects of nutrition mediums (MS and VW), BAP, organic additives on shoot growth (via number of leaves) and root growth (via root number and root length)

Results: In summary, in Experiment 1, most parameters recorded to evaluate Phalaenopsis root growth performed better in 1/2xMS medium. In experiment 2, 1/2xMS + 0.05 mg/L BAP showed the highest number of roots per shoot and 1/2xMS medium and VW medium without BAP showed the longest roots length. In experiment 3, VW + potato showed the highest number of leaves per shoot and VW + CW showed the longest roots length. In experiment 4, most metrics showed that Phalaenopsisshoot and root growthwere better in VW + CW.

Conclusion: Both 1/2xMS and VW medium are necessary for shoot from hybrid-seeds growth in the first 6-weeks and VW medium is favored for development of shoot and root after 6-weeks

Key words: Phalaenopsis, growth regulator, organic additives, coconut water, potato, banana, peptone

1 INTRODUCTION

Darwin's request of J. D. Hooker to "Have pity on me and let me write once again on Orchids for I am in transport of admiration" [1]. This quote from the England biologist Charles Robert Darwin undoubtedly demonstrates how unique and fascinating this flower is. With more than 800 identified genera and 25,000 species, orchids are one of the flowering plant groups with the greatest diversity. Orchids are admired for their exquisite, long-lasting blossoms, which come in a staggering variety of sizes, shapes, and colors. Nowadays, cultivating orchids is more than just a pastime; it is a global industry that accounts for 8% of global floriculture trade and has the power to change a nation's economic climate. Orchids are one of the

top ten cut flowers thanks to extensive tissue culture-based hybridization of beautiful and rare varieties [2].

In addition to having significant decorative and the commercial value, orchids also hold distinct cultural significance. Together with the Chinese plum, chrysanthemum, and bamboo, the orchid is one of the "Four Gentlemen Among the Flowers" in classical Chinese literature [3]. Phalaenopsis (moth orchids), one of the most popular types of orchids, are prized for their particularly attractive and long-lasting flowers and the simplicity of growing in artificial surroundings. So, Phalaenopsis orchids are now among the most expensive potted decorative plants in existence. The Floriculture Crops Report published by the U.S. Department of Agriculture (USDA) estimates that potted orchids generated $191 million in wholesale sales in 2011 and projects a 15% annual value growth. 70% of these orchids were Phalaenopsis imported from Taiwan [4]. Phalaenopsis (Orchidaceae) is a genus of about 40 species found in the Himalayas, China, Tibet, Southeast Asia, Formosa, the Philippines, the Andaman Islands, Sumatra, Java, Borneo, Celebes, New Guinea, and northern Australia [5]

As a monopodial plant, phalaenopsis is normally multiplied by cutting or separating offshoots; however, these methods have a low rate of multiplication and restrict the growth of the mother plant, making them unsuitable for large-scale production. Because of this, it is difficult for them to reproduce vegetatively, and their seedlings' characteristics vary. The fact that Phalaenopsis takes at least three years to flower in a greenhouse is one of the main problems with commercial production of the plant. Tissue culture may therefore be a helpful alternative strategy for propagating orchid species [6]. However, different plant species, explant types, and culture conditions have varying degrees of micropropagation efficacy [7]. The presence of a growth regulator and an organic addition provider in the medium play a significant role in the effectiveness of plant tissue culture in enhancing orchid vigor [8]. Similar to other orchids, several researchers have introduced and developed different culture media and plant growth regulators for Phalaenopsis orchid shoot regeneration *in vitro*[9]. Aside from salt compositions, vitamins, carbon sources, and growth regulators, another significant component added to orchid propagation media are compounds known as organic additives. Organic additives such as coconut water (CW), banana, potato, and peptone have been shown to significantly improve orchid germination and micropropagation[10].

The effects of a combination with organic additives such as coconut water (CW), banana, potato, peptone, and BAP (6-benzylaminopurine) concentration supplemented to MS medium [11], half-strength MS medium (1/2xMS medium), VW medium [12] and half-strength VW medium (1/2xVW medium) and vitamin Morel [13} on the growth of Phalaenopsisshoot derived from hybrid-seeds has rarely studied. As a result, the current study was carried out to assess the effects of culture media, BAP, and organic additives on shoot regeneration from Phalaenopsisshoots derived from hybrid-seeds;thereby, contributing micropropagation of wild Phalaenopsis of Vietnamforconservation and development task.

2. MATERIALS AND METHODS

2.1 Plant materials:

*Materials:*Hybrid-seeds of Phalaenopsis was cultured on MS medium and shoots derived from hybrid-seeds having 2 month-age after germination were used in all experiments. Hybrid-seeds was sourced fromwild forest of Western High-land Region in Lam Dong province of Vietnam. The initial shoot samples were healthy and disease-free *in vitro.*Phalaenopsis orchid shoot wasstudied in the Plant Biotechnology Laboratory, belonged to International University Ho Chi Minh City.

*Media:*MS [11] and VW [12] basic nutrient mediumsand vitamin Morel [13] supplemented with 20 g/L sucrose,10% coconut water (CW), activated charcoal 2 g/L, and 8 g/L agar and adjusted to pH = 5.8-6 before sterilization. Media was supplemented with plant growth regulators: BAP (6-Benzylaminopurin) sourced from Sigma Co.; and organic additives: banana (50 g/L), potato (50 g/L), peptone (1 g/L)

*Culture conditions:*Medium was sterilization at 121^{o}C, 1 atm for 20 minutes. Light schedule was set up 16 hours of light/ 8 hours of darkness, light intensity 37.04 µmol/s/m^{2}, temperature 26 ± 2^{o}C

Organic additives preparation: CW were extracted directly from young coconuts and filtered once to remove residues. Potato was prepared as follows: potato was selected based on smoothness and freshness, then washed, peeled, cut into small pieces, and weighed 50 g/L media, then graunnd, cooked and filtered before adding to the culture medium. Bananas were prepared as follows: bananas were peeled, chopped, and weighed 50 g/L medium, thengraund and cooked before adding to the culture medium.

2.2 Methods

2.2.1. Effects of media on shoot regeneration of Phalaenopsis. Shoot explants were cultured on medium of:(MS medium, 1/2xMS medium, VW medium, 1/2xVW medium), respectively.

2.2.2. Effect of BAP concentration, 1/2xMS andVW media on shoot regeneration of Phalaenopsis. Shoot explants were cultured on medium of:1/2xMS + BAP (0-05-0.1 mg/L), VW + BAP (0-0.05-0.1 mg/L).

2.2.3. Effect of media and organic additives on shoot regeneration of Phalaenopsis. Shoot explants were cultured on medium of: 1/2xMS (CW-potato-banana-peptone), VW (CW-potato-banana-peptone).

2.2.4. Effect of VW medium, 0.05 mg/L BAP with organic additives on shoot regeneration of Phalaenopsis. Shoot explants were cultured on medium of: VW + BAP (0.05 mg/L) + CW (10%) + (potato-banana-peptone).

2.3 Data analysis

Experiment was laid out in a completely randomized design (CRD). Each treatment was consisted of 4 replicates. Each replicates was carried out with 4 bottles (350 ml). Each bottle was contained 5 shoots. Data was collected after 12 weeks. Data were analyzed by one-way ANOVA followed with a Duncan testat P = 0.05 using SPSS software (IBM SPSS Statistics 23). Monitoring indicators: Number of leaves, number of roots, length of roots (cm)

3. RESULT

3.1Effects of media on shoot regeneration of Phalaenopsis

3.1.1 Shoot growth

Finding the optimal media for the high rate of plant growth, good quality shoots without mutating, and rapid development of leaves and roots before transferring to the complete plant medium is essential in *in vitro* production. At 6 weeks and 12 weeks, there was no significant difference among all the treatments in the number of leaves developed on each shoot (Table1 and Figure 1A,B). Since the shoots were too little and freshly developed, thus the growth rate was still quick. As a result, the data is nearly identical with no differences. Since there was no significant differences in the different culture media, comparisons of the growth rates of leaves per shoot of each culture medium over periods were carried out (Figure 1 C, D, E, F)

The growth rate of the number of leaves per shoot of each culture medium at different periods is significantly different(Table 1). In which the growth rate of leaves per shoot of 1/2xMS medium was slightly higher than that of MS medium and 1/2xVW medium was slightly better than VW medium because, from week 10, it showed outstanding growth and was close to the height in the case of MS medium and higher than in the case of VW medium. After 12 weeks, the number of leaves per shoot on1/2xMS medium increased from 3.45 to 4.50. Meanwhile, VW medium had the lowest growth rate of the number of leaves per shoot.

Overall, both the number of leaves per shoot and the growth rate of the number of leaves per shoot on 1/2xMS medium were the most optimal across the greatest number of recorded parameters.

3.1.2 Root growth

A look at root length cultured on different culture media showed that after 12 weeks, 1/2xVW medium induced the highest root length in cultured explants (4.97cm) and was significantly different from the remaining treatments (Table 2 and Figure 2 A, B). In terms of number of roots per shoot, after 12 weeks, there was no significant difference among all the treatments (Figure 2 A, B).

In summary, among all treatments in experiment 1, the treatment for robust leaf was 1/2xMS medium and the treatment for highest number of roots and root length was 1/2xVW. However, the treatment for high number of roots per shoot and high root length also was 1/2xMS medium (2.95 roots/shoot and 4.06 cm). So, in four basal culture media, 1/2xMS medium would be an appropriate basal medium for shoot regeneration of phalaenopsis.Figure 3 shows the morphological characteristics of shoots, roots and leaves of Phalaenopsis effects of MS medium, 1/2xMS medium, VW medium and 1/2xVW medium. Figures 3D and 3H clearly show vigorous root growth after 12 weeks

Table 1. Effects of different culture medium on number of leaves per shoot of Phalaenopsis

Medium	No. of leaves per shoot					
	2 (weeks)	4	6	8	10	12
MS	1.65 ± 0.15	2.20 ± 0.18	2.45 ± 0.26	3.30 ± 0.37	3.95 ± 0.50	4.60 ± 0.76
1/2xMS	1.55 ± 0.25	2.15 ± 0.17	2.40 ± 0.16	2.90 ± 0.17	3.45 ± 0.32	4.50 ± 0.40
VW	1.55 ± 0.10	2.10 ± 0.33	2.25 ± 0.26	2.75 ± 0.33	2.95 ± 0.26	3.40 ± 0.29
1/2xVW	1.25 ± 0.21	1.75 ± 0.30	1.75 ± 0.13	2.35 ± 0.25	3.25 ± 0.22	3.75 ± 0.29

Table2. Effects of different culture medium on number of roots per shoot and root length of Phalaenopsis after 12 weeks

Medium	No. of roots per shoot	Root length (cm)
MS	2.50 ± 0.31	2.10b ± 0.06
1/2x MS	2.95 ± 0.64	4.06a ± 0.22
VW	2.90 ± 0.29	4.19a ± 0.59
1/2xVW	3.20 ± 0.37	4.97a ± 0.50

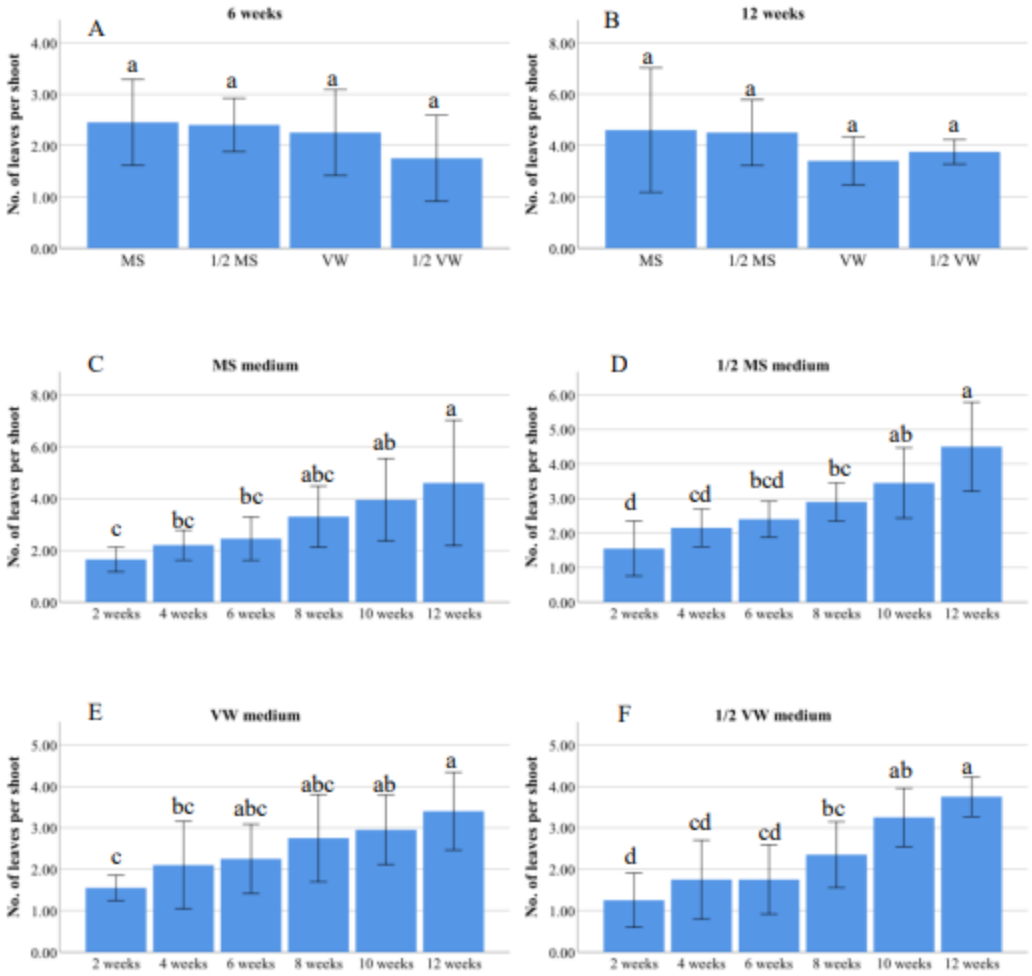

Figure 1.(A, B) Effects of different culture media on number of leaves per shoot of Phalaenopsis after 6 weeks and 12 weeks. (C, D, E, F) Growth rate of number of leaves per shoot of each culture medium over weeks. Treatments with different letters (a, b, c…) in the same bar chart differ significantly ($p < 0.05$). The bars represent mean ± standard error of the mean (SEM).

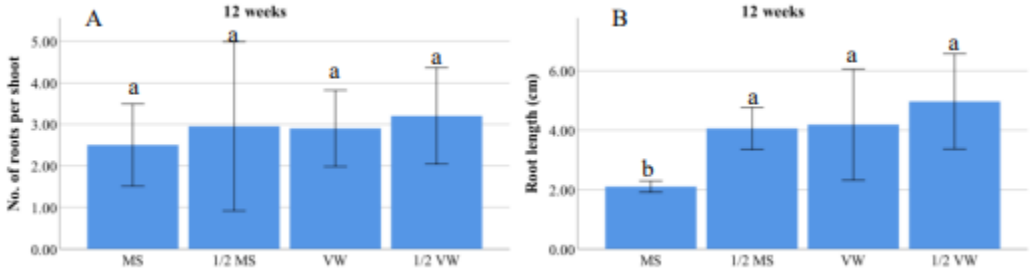

Figure 2.(A, B) Effects of different culture media on number of roots per shoot and root length of Phalaenopsis after 12 weeks. Treatments with different letters (a, b, c…) in the same bar chart differ significantly ($p < 0.05$). The bars represent mean ± standard error of the mean (SEM).

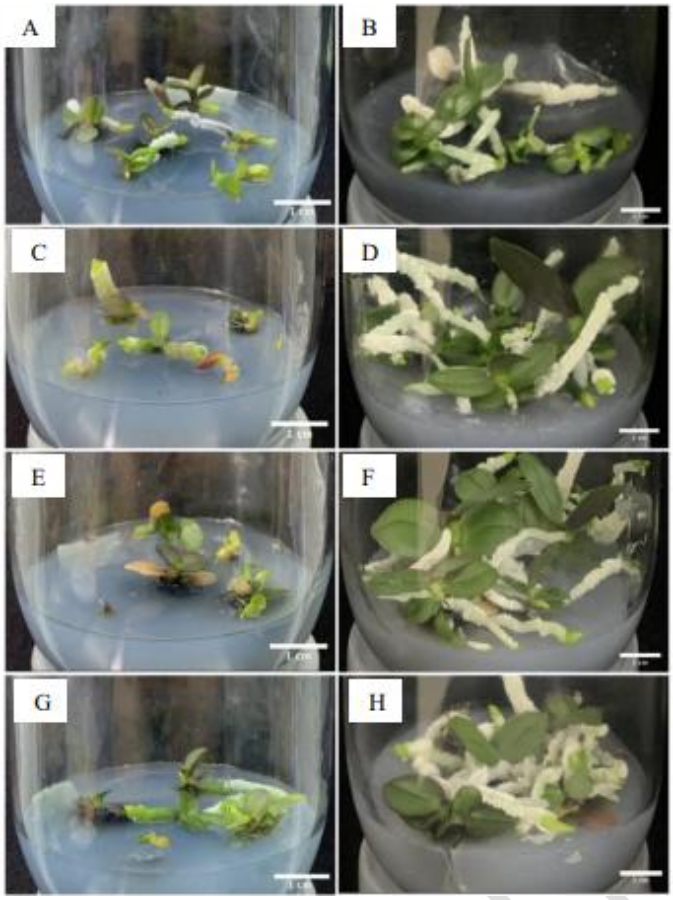

Figure 3. Effects of (A, B) MS medium, (C, D) 1/2xMS medium, (E, F) VW medium and (G, H) 1/2xVW medium on number of leaves per shoot, number of roots per shoot and root length of Phalaenopsis after 6 weeks and 12 weeks. After 6 weeks (A, C, E, G) and after 12 weeks (B, D, F H). Bar = 1 cm.

3.2 Effect of BAP concentrations, 1/2xMS and VW medium on shoot regeneration of Phalaenopsis

3.2.1 Shoot growth

In experiment 2, different concentrations of BAP were added to the two culture media including 1/2xMS medium and VW medium to see if the number of leaves per shoot, the number of roots per shoot, and the root length depended on culture medium, and BAP concentration.

In terms of the number of leaves per shoot, no significant differences was observed for the effects of different BAP concentrations with different culture media (Tabie3 and Figure 4 A, B). The growth rate of the number of leaves per shoot of different BAP concentration with different culture media at different periods is significantly different (Figure 4 C, D, E, F, G, H). Specifically, the growth rate of leaves per shoot of 1/2xMS medium without BAP was higher than that of 1/2xMS + 0.05 mg/L and 1/2xMS + 0.1 mg/L BAP and VW+ 0.1 mg/L BAP was slightly better than VW+ 0.05 mg/L BAP. Medium of 1/2xMS

without BAP had the highest growth rate of leaves per shoot, increasing 3 times from 2 weeks to 12 weeks. Meanwhile, VW + 0.05 mg/L BAP had the lowest growth rate of the number of leaves per shoot

3.2.2 Root growth

In terms of roots development, both groups of number of roots per shoot and roots length showed significant differences between control treatment and treatments with different BAP concentration after 12 weeks (Table 4 and Figure 5 A, B). In the case of 1/2xMS medium, treatment 1/2xMS + 0.05 mg/L BAP produced the high number of roots (4.80 roots/shoot). Treatment 1/2xMS without BAP, on the other hand, had the best root length (4.06a ± 0.22 cm). In the case of VW medium, the VW + 0.05 mg/L BAP yields more roots/shoot than the treatments with VW + 0.1 mg/L BAP and VW without BAP. In comparison, the VW without BAP produced longer roots (4.18cm).

In summary, experiment 2, the treatment for high number of roots was 1/2xMS + 0.05 mg/L BAP and roots length was 1/2xMS medium without BAP. Conversely, 1/2xMS medium without BAP gave the least number of roots per shoot and 1/2xMS + 0.05 mg/L BAP had the shortest root length. However, to achieving the best cost-to-quality ratio, 1/2xMS medium without BAP would be an appropriate basal medium for large-scale tissue culture of Phalaenopsis. Figure 6 shows the morphological characteristics of shoots, roots and leaves of Phalaenopsis under effects of 1/2xMS + 0.05 mg/L BAP, 1/2xMS + 0.1 mg/L BAP, VW + 0.05 mg/L BAP and VW + 0.1 mg/L BAP, 1/2xMS medium, VW medium. Figures 6K and 6M clearly show vigorous root growth after 12 weeks

Table 3. Effects of different BAP concentration with different culture medium on number of leaves per shoot of Phalaenopsis

Medium	BAP (mg/L)	No. of leaves per shoot					
		2 (weeks)	4	6	8	10	12
1/2xMS	0.05	1.25 ± 0.36	1.40 ± 0.30	1.80 ± 0.36	2.25 ± 0.41	2.70 ± 0.42	3.25 ± 0.52
	0.1	1.20 ± 0.14	2.00 ± 0.18	2.40 ± 0.14	2.70 ± 0.17	2.75 ± 0.19	3.2 ± 0.08
	0.0	1.55 ± 0.25	2.15 ± 0.17	2.40 ± 0.16	2.90 ± 0.17	3.45 ± 0.32	4.5 ± 0.40
VW	0.05	1.40 ± 0.14	2.00 ± 0.16	2.35 ± 0.31	2.60 ± 0.36	2.80 ± 0.34	2.95 ± 0.36
	0.1	1.55 ± 0.05	2.10 ± 0.19	2.40 ± 0.23	2.8 ± 0.35	3.25 ± 0.45	3.85 ± 0.66
	0.0	1.55 ± 0.09	2.10 ± 0.33	2.25 ± 0.26	2.75 ± 0.33	2.95 ± 0.26	3.40 ± 0.29

Table4. Effects of different BAP concentration with different culture medium on number of root per shoot and root length of Phalaenopsis after 12 weeks

Medium	BAP (mg/L)	No. of roots per shoot	Root length (cm)
1/2xMS	0.05	4.80a ± 0.67	2.70b ± 0.09
	0.1	3.90ab ± 0.40	2.66b ± 0.13
	0.0	2.95ab ± 0.64	4.06a ± 0.22
VW	0.05	3.60ab ± 0.22	3.79ab ± 0.15
	0.1	2.00b ± 0.72	3.45ab ± 0.14
	0.0	2.90ab ± 0.29	4.18a ± 0.59

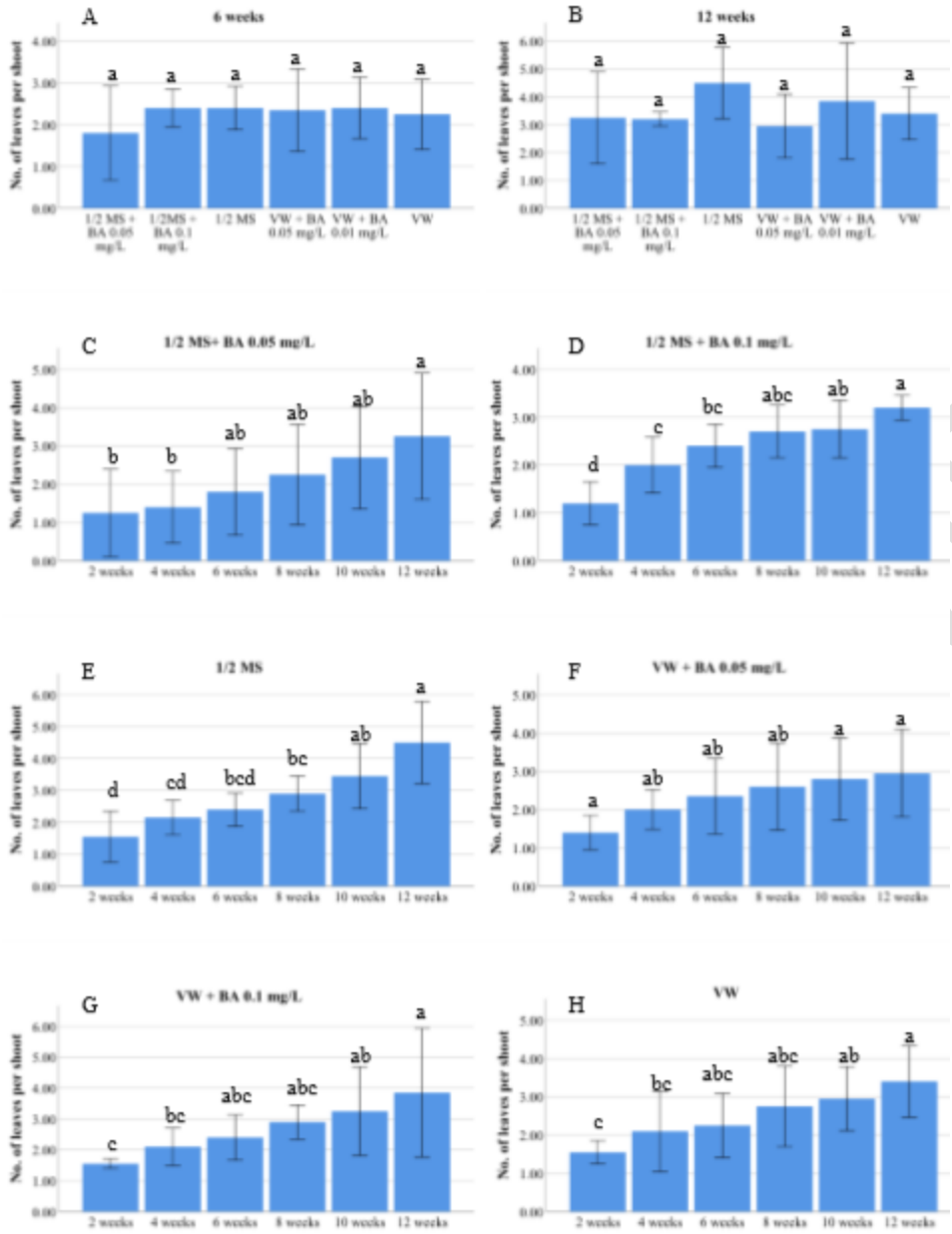

Figure 4.(A, B) Effects of different BAP concentration with different culture media on number of leaves Phalaenopsis after 6 weeks and 12 weeks. (C, D, E, F, G, H) Growth rate of number of leaves per shoot of different BAP concentration with different culture media over weeks. Treatments with different letters (a, b, c…) in the same bar chart differ significantly ($p < 0.05$). The bars represent mean ± standard error of the mean (SEM)

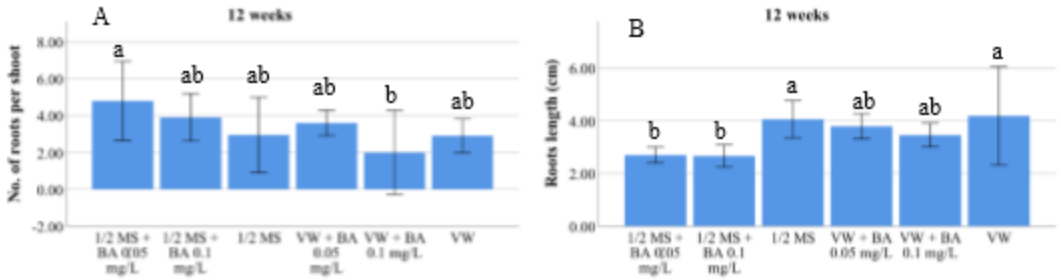

Figure 5. (A, B) Effects of different BAP concentration with different culture media on number of roots per shoot and root length of Phalaenopsis after 12 weeks. Treatments with different letters (a, b, c…) in the same bar chart differ significantly ($p < 0.05$). The bars represent mean ± standard error of the mean (SEM).

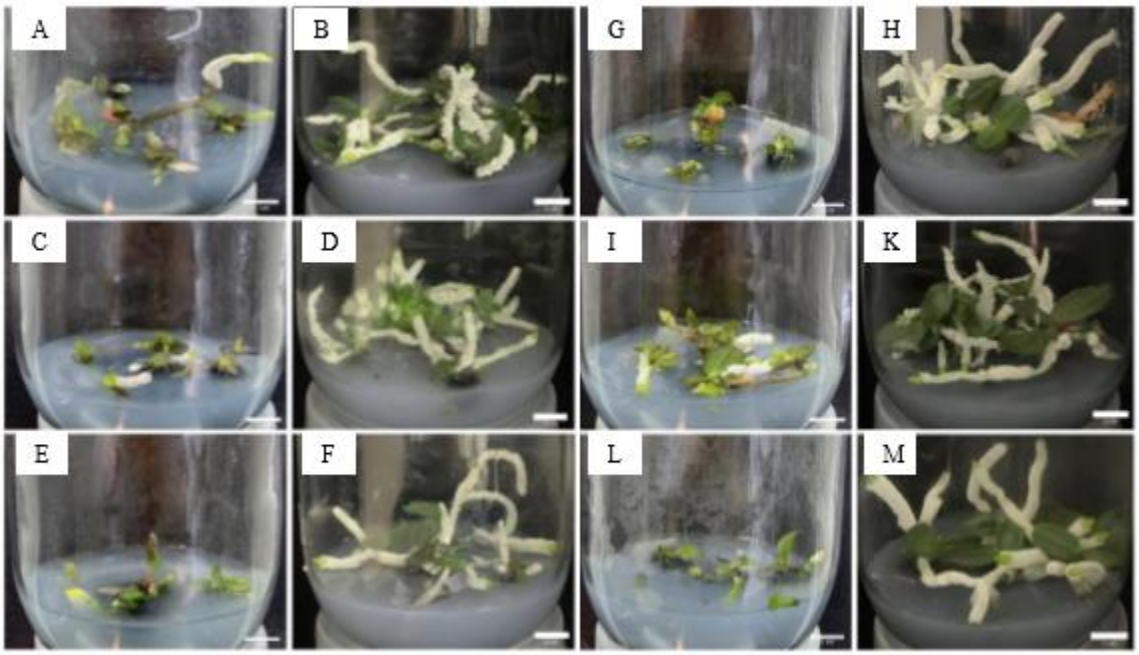

Figure 6. Effects of (A, B) 1/2xMS + 0.05 mg/L BAP, (C, D) 1/2xMS + 0.1 mg/L BAP, (E, F) VW + 0.05 mg/L BAP and (G, H) VW + 0.1 mg/L BAP, (I, K) 1/2xMS medium, (L, M), VW medium on number of leaves per shoot, number of roots per shoot and root length of Phalaenopsis after 6 weeks and 12 weeks. After 6 weeks (A, C, E, G, I, L) and after 12 weeks (B, D, F, H, K, M). Bar = 1 cm.

3.3 Effect of media and organic additives on shoot regeneration of Phalaenopsis

3.3.1 Shoot growth

In experiment 3, Table 5 show that the number of leaves per shoot cultured on different organic additives with different culture media showed that after 6 weeks and after 12 weeks, CW showed a significantly lower response for leaf growth compared to banana and peptone treatments, which gave statistically similar results. In contrast, when 1/2xMS + potato and VW + potato increase significantly the number of leaves per shoot gave 8.95 after 12 weeks.

3.3.2 Root growth

In terms of the number of roots per shoot, all treatment showed no significant differences after 12 weeks(Table 6). In constrast, different organic additives caused significant differences in root length. In 1/2xMS + banana exhibited the longest roots length (3.62 cm) and 1/2xMS + potato came lowest roots length (2.42 cm).

In VW medium, CW showed the significantly longest root length (4.78 ± 0.09 cm) while potato also gave the lowest value (2.55 cm). In addition, VW + banana also had a significantly high number of roots/shoots as well as root length (3.35 roots/shoot and 4.14 cm)(Figure 7).

Figure 8 shows the morphological characteristics of shoots, roots and leaves of Phalaenopsis under effects of 1/2xMS + CW, 1/2xMS + potato, 1/2xMS + banana, 1/2xMS + peptone, and VW + CW, VW + potato, VW + banana, VW + peptone. Figures 8, clearly show vigorous leaf growth and Figure 6K clearly show vigorous root growth after 12 weeks.

Table5. Effects of different organic additives with different culture medium on number of leaves per shoot of Phalaenopsis over weeks

Medium	Organic additives (g/L)	No. of leaves per shoot					
		2 (weeks)	4	6	8	10	12
1/2xMS	CW (10%)	0.85 ± 0.40	1.45 ± 0.43	1.65b ± 0.50	2.30b ± 0.42	3.90 ± 0.70	5.40ab ± 0.77
	Potato (50)	1.95 ± 0.05	2.45 ± 0.22	2.85ab ± 0.31	3.25ab ± 0.38	4.95 ± 1.03	6.8ab ± 1.22
	Banana (50)	1.95 ± 0.09	2.30 ± 0.13	2.55ab ± 0.09	3.25ab ± 0.15	4.25 ±1.03	5.20b ± 0.49
	Peptone (1)	1.90 ± 0.10	2.4 ± 0.18	2.90ab ± 0.17	3.30ab ± 0.13	4.15 ± 0.15	5.10b ± 0.42
VW	CW (10%)	1.85 ± 0.31	2.40 ± 0.18	2.85ab ± 0.29	3.50ab ± 0.21	4.55 ± 0.50	5.20b ± 0.55
	Potato (50)	1.70 ± 0.13	2.55 ± 0.21	3.10a ± 0.06	4.55a ± 0.69	6.15 ± 0.87	8.95a ± 1.11
	Banana (50)	1.60 ± 0.34	2.00 ± 0.29	2.45ab ± 0.21	3.25ab ± 0.22	4.65 ± 0.35	6.30ab ± 0.58
	Peptone (1)	1.65 ± 0.56	2.25 ± 0.42	2.55ab ± 0.32	3.10ab ± 0.10	4.80 ± 0.22	6.10ab ± 0.64

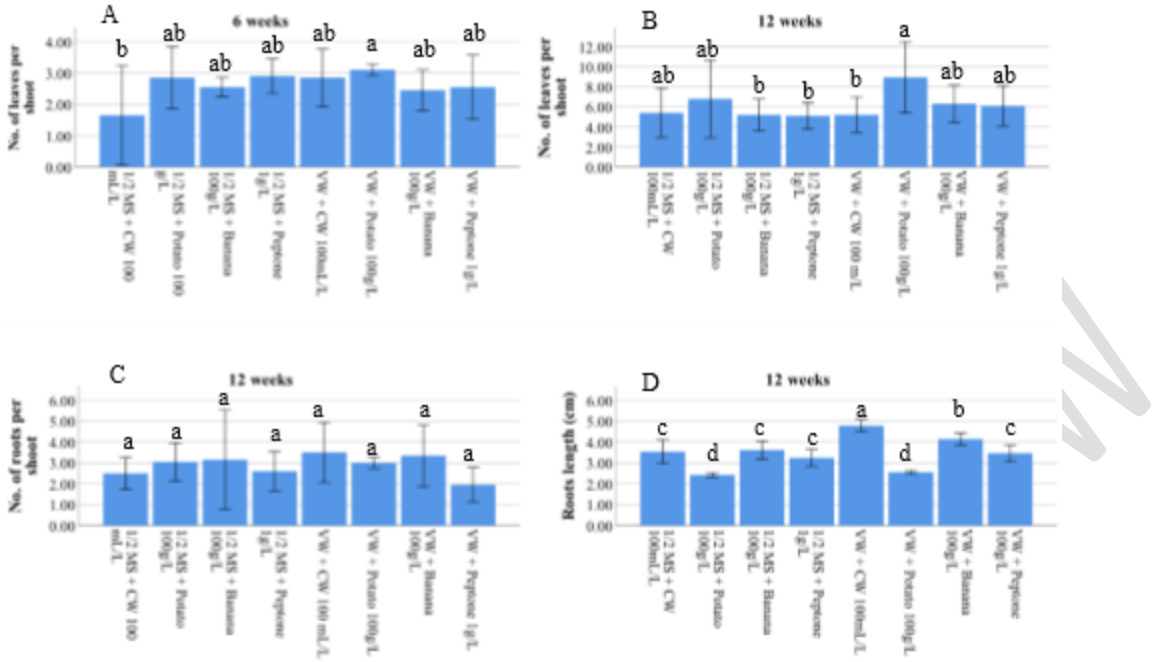

Figure 7. (A, B) Effects of different organic additives with different culture media on number of leaves per shoot after 6 weeks and 12 weeks, and (C, D) on number of roots per shoot and root length after 12 weeks of Phalaenopsis. Treatments with different letters (a, b, c…) in the same bar chart differ significantly ($p < 0.05$). The bars represent mean ± standard error of the mean (SEM).

Table6. Effects of different organic additives with different culture medium on number of roots per shoot and root length of Phalaenopsis after 12 weeks

Medium	Organic additives (g/L)	No. of roots per shoot	Root length (cm)
1/2xMS	CW (10%)	2.50 ± 0.24	3.53c ± 0.18
	Potato (50)	3.05 ± 0.29	2.42d ± 0.03
	Banana (50)	3.15 ± 0.75	3.62c ± 0.14
	Peptone (1)	2.60 ± 0.29	3.24c ± 0.13
VW	CW (10%)	3.50 ± 0.45	4.78a ± 0.09
	Potato (50)	3.00 ± 0.08	2.55d ± 0.03
	Banana (50)	3.35 ± 0.46	4.14b ± 0.09
	Peptone (1)	1.95 ± 0.26	3.46c ± 0.13

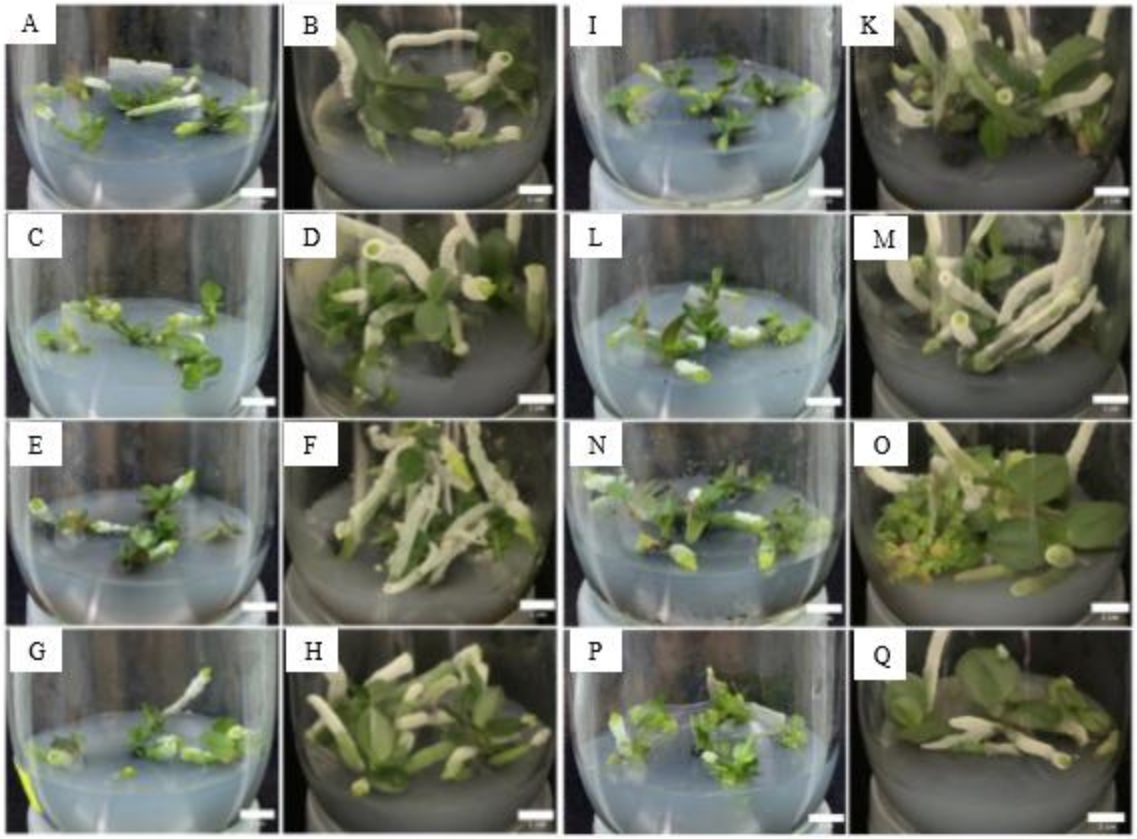

Figure 8. Effects of (A, B) 1/2xMS medium + CW, (C, D) 1/2xMS medium + potato, (E, F) VW 1/2xMS medium + banana, (G, H) 1/2xMS medium + peptone, and (I, K) VW medium + CW, (L, M) VW medium + potato, (N, O) VW medium + banana, (P, Q) VW medium + peptone on number of leaves per shoot, number of root per shoot and root length of Phalaenopsis after 6 weeks and 12 weeks. After 6 weeks (A, C, E, G, I, L, N, P) and after 12 weeks (B, D, F, H, K, M, O, Q). Bar = 1 cm

3.4 Effect of VW medium, 0.05 mg/L BAP with organic additives on shoot regeneration of Phalaenopsis

3.4.1 Shoot growth

In experiment 4;There was no significant difference in the number of leaves per shoot of the combination of VW medium, 0.05 mg/L BAP with different organic additives (Table 7 and Figure 9 A, B). Figure 9 (C, D, E, F) showed that the growth rate of the number of leaves per shoot of the combination of VW medium, 0.05 mg/L BAP with different organic additives at different periods differs significantly. It was established that after 12 weeks, the combination of VW medium + 0.05 mg/L BAP + CW + potato had the highest growth rate of leaves per shoot, increasing 6 times from 2 weeks to 12 weeks. This was in agreement with experiment 3, where 1/2xMS + potato (6.8 leaves per shoot) and VW + potato (8.9 leaves per shoot) had an effect on leaf growth(Table 5). Meanwhile, the combination of VW + 0.05 mg/L BAP+ CW + banana + potato had the lowest growth rate of the number of leaves per shoot (Table 7)

3.4.2 Root growth

The results observed for the number and length of roots in reference to treatment with of the combination of VW + 0.05 mg/L BAP + organic additives are shown in Figure 10 (A, B) . The combination of different organic additives with VW + 0.05 mg/L BAP caused significant differences in root length as well as number of roots per shoot. The medium of VW + 0.05 mg/L BAP+ CW (Table 8)showed significant highest in both roots length (2.69 cm) and number of roots per shoot (4.80 roots per shoot) while VW + 0.05 mg/L BAP + CW + potato was reverse. This was in agreement with experiment 3 in this study, where CW supplementation had an effect on root growth

Figure 11 shows the morphological characteristics of shoots, roots and leaves of Phalaenopsis under effects of VW + 0.05 mg/L BAP+ CW, VW + 0.05 mg/L BAP+ CW + banana, VW + 0.05 mg/L BAP+ CW + potato, and VW + 0.05 mg/L BAP + CW + banana + potato. Figures 11B clearly show vigorous root growth after 12 weeks

Table 7. Effects of the combination of VW medium, 0.05 mg/L BAP with different organic additives on number of leaves per shoot of Phalaenopsis over weeks

Organic additives	No. of leaves per shoot					
	2 (weeks)	4	6	8	10	12
CW	0.85 ± 0.40	1.50 ± 0.44	1.70 ± 0.51	2.50 ± 0.39	3.90 ± 0.70	5.40 ± 0.77
CW+Banana	1.5 ± 0.19	2.1 ± 0.24	2.95 ± 0.26	3.5 ± 0.29	4.55 ± 0.13	5.55 ± 0.30
CW +Potato	1.15 ± 0.26	2.10 ± 0.34	3.05 ± 0.71	3.75 ± 0.66	5.95 ± 0.97	7.65 ± 1.26
CW-Ban+Pot	1.45 ± 0.22	1.95 ± 0.10	2.50 ± 0.17	3.00 ± 0.16	3.80 ± 0.34	4.70 ± 0.50

Table 8. Effects of the combination of 1/2xMS medium, 0.05 mg/L BAP with different organic additives on number of roots per shoot and root length of Phalaenopsis after 12 weeks

Organic additives	No. of roots per shoot	Root length (cm)
CW	4.80a ± 0.67	2.69a ± 0.09
CW +Banana	2.90b ± 0.13	2.59a ± 0.10
CW+Potato	2.65b ± 0.19	2.28ab ± 0.11
CW+Banana+Potato	3.00b ± 0.24	2.14b ± 0.09

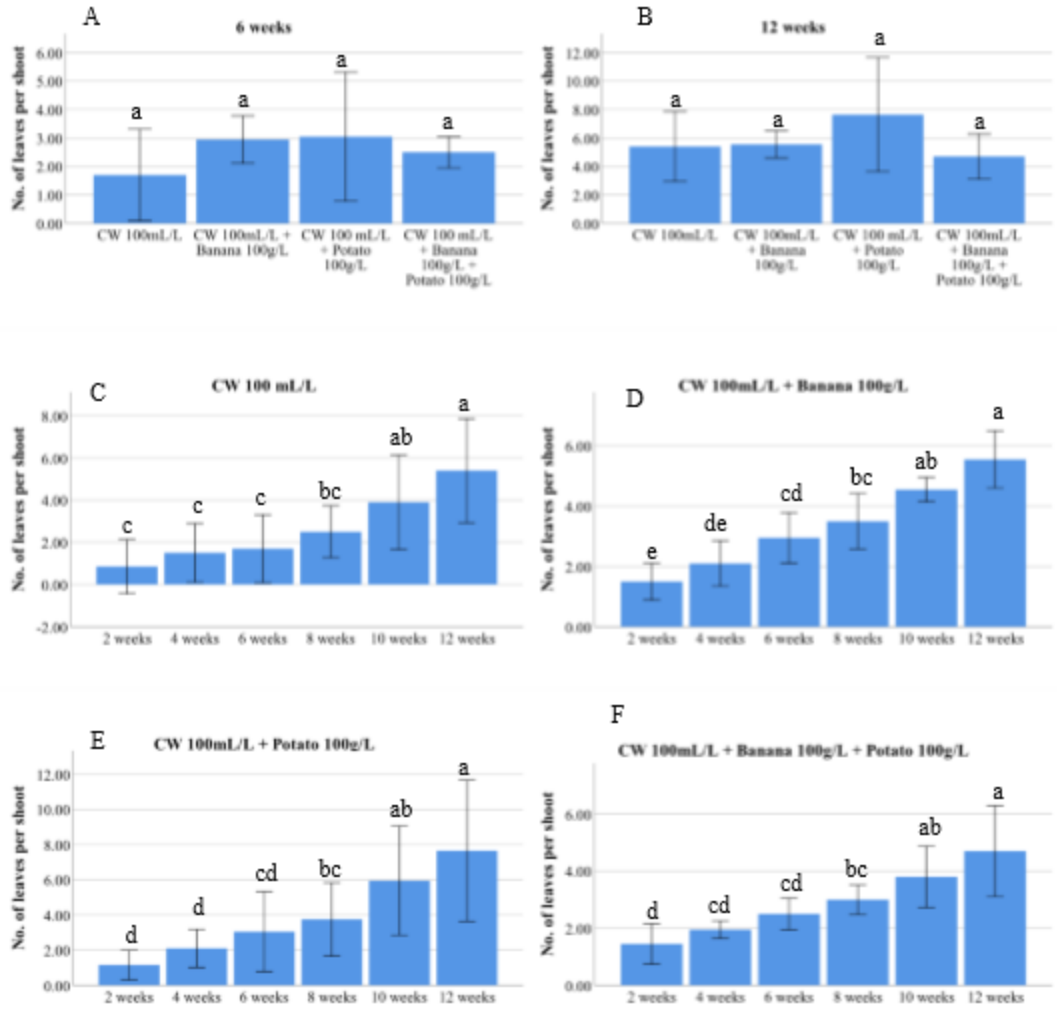

Figure 9.(A, B) Effect of the combination of 1/2xMS medium + 0.05 mg/L BA with different organic additives on number of leaves per shoot after 6 weeks and 12 weeks. (C, D, E, F) Growth rate of number of leaves per shoot of the combination of ½xMS medium + 0.05 mg/L BA with different organic additives. Treatments with different letters (a, b, c…) in the same bar chart differ significantly ($p < 0.05$). The bars represent mean ± standard error of the mean (SEM).

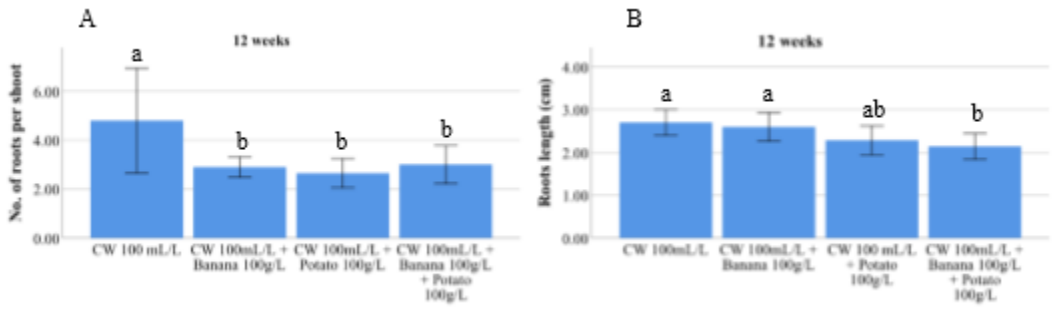

Figure 10.(A, B) Effects of the combination of ½xMS medium + 0.05 mg/L BA with different organic additives on number of roots per shoot and root length of Phalaenopsis after 12 weeks. Treatments with different letters (a, b, c…) in the same bar chart differ significantly ($p < 0.05$). The bars represent mean ± standard error of the mean (SEM).

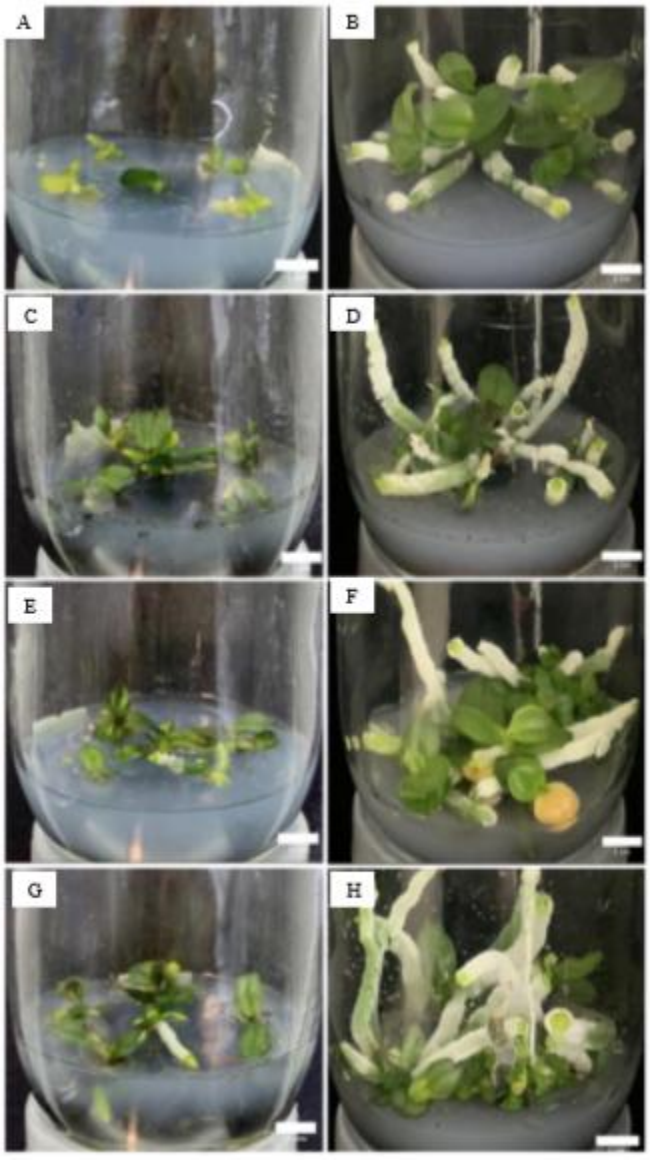

Figure 11. Effects of (A, B) VW+ 0.05 mg/L BAP+ CW, (C, D) VW+ 0.05 mg/L BAP + CW + banana, (E, F) VW+ 0.05 mg/L BAP+ CW + potato, and (G, H) VW+ 0.05 mg/L BAP+ CW + banana + potato on number of leaves per shoot, number of roots per shoot and root length of Phalaenopsis after 6 weeks and 12 weeks. After 6 weeks (A, C, E, G) and after 12 weeks (B, D, F H). Bar = 1 cm.

4. DISCUSSION

The influence of varied medium strengths on plant organogenesis *in vitro* could be significant. The nutrients in media required for effectiveness of culture may fluctuate depending on plant species and genotype [14]. Tetsumura et al. [15] also discovered that decreasing the strength of MS medium leading increasing*in vitro* shoot and root production in highbush blueberry (*Vacciniumcorymbosum* and *V. virgatum*) and reduction of MS medium strength increased the callus, shoot and root formation in *Vacciniumcorymbosum*.

In several orchid tissue cultures, such as *Dendrobium*[16], *Cymbidium pendulum*[17], and *Cypripedium macranthos*[18], an organic additive, one of a crucial medium's elements, has been employed to encourage growth, development, and enhance shoot regeneration. The addition of CW to the VW medium, together with potato and banana homogenate, had a significant impact on shoot regeneration and plantlet development. Organic growth additives can promote the production and multiplication of *B. Dhaninivatii* shoots [19]. Minea et al. (2004)[20] studied the impact of 10% banana homogenate (BH) on the size of *Spathoglottiskimballianai*leaves. Gnasekaran[21] investigated the effects of organic potato, papaya, and tomato extracts of varying compositions on orchid PLBs of *V. Kasem's Delight* species in *in vitro* orchid propagation. Coconut water(CW) content compounds having action same as cytokinin and auxin that affect plant growth and development. Auxin plays a role in the transmission of environmental cues like light and gravity as well as the control of the branching mechanisms in roots and shoots[22]. Also, due to its growth-regulating qualities and cytokinin activity, which encourages cell division and promotes rapid growth, CW has been employed in tissue culture techniques [23]. Peptone has been used as a component to accelerate the growth of plant tissue *in vitro* in large plants like *Perseaamericana's* shoot and root regeneration (Nhut et al. 2008) [24]. Peptone is also reported to have aided Phalaenopsis hybrid seed germination *in vitro* and the development of protocorm-like bodies [25]. Moreover, it promoted *Calopogontuberosus* seed germination and improved protocorm growth [26].

This finding is consistent with previous studies on the growth and development of *in vitro* shoots of *Bulbophyllumdhaninivatii*Seidenf[19]. Kongbangkerdet al [19]examined *B. dhaninivatii* shoots cultivated in vitro for 12 weeks on semisolid VW medium, which was supplemented with different concentrations of a mixture of coconut water, potato extract (PE), and banana homogenate (BH). They stated that adding organic supplements to the medium resulted in higher *in vitro* shoot development and morphological improvement over those cultures. The culture media with the highest concentration of CW (150 ml/L) and potato extract (50 g/L) produced the greatest number of shoot regeneration. This also indicates that the number of leaves per shoot is the greatest.

Shoot initiation and proliferation are accelerated when organic additives are added to culture media, either singly or in combination. For *in vitro* cultures to succeed, different kinds and amounts of organic nutrients must be used, depending on the species and genotype [28]. A number of research were done to determine how organic additions affected shoot growth, regeneration, and rhizogenesis, which in turn produced healthy plantlets. [27, 17, 28].

5. CONCLUSION

The task of determining the effect of culture medium, growth regulators, and organic additives on shoot regeneration from shoot derived from hybrid-seeds of Phalaenopsis is the right way to apply to hybrid-seeds of Phalaenopsis, promoting the development of rare Phalaenopsisin Vietnam. This is necessary to

proactively source high-quality, disease-free seedlings for product development for both domestic consumption and export.

In Experiment 1, after 12 weeks, the number of leaves per shoot on 1/2xMS medium increased from 3.45 to 4.50; and root growth performed better in 1/2xMS medium (4.06 cm). In experiment 2, 1/2xMS medium + 0.05 mg/L BAP showed the highest number of roots per shoot (4.80 roots per shoot). 1⁄2xMS medium and VW medium without BAP showed the longest roots length (4.06 cm and 4.18 cm, respectively). In experiment 3, VW medium + potato showed the highest number of leaves per shoot (8.95 leaves per shoot) and VW medium + coconut water (CW) showed the longest roots length (4.78 ± 0.09 cm). in experiment 4, root growth performed better in medium enriched with CW (4.80 roots per shoot and root length 2.69 cm).

REFERENCES

[1] Burkhardt, Frederick et al. (eds)*The Correspondence of Charles Darwin*. Vol. 13 (Suppl). Cambridge University Press, Cambridge, 2003

[2] Samira Chugh, SatyakamGuha, and I. Usha Rao. Micropropagation of Orchids: A Review on the Potential of Different Explants.*ScientiaHorticulturae*2009, 122(4):507–20.

[3] Li Chengru, Na Dong, Yamei Zhao, Shasha Wu, Zhongjian Liu, and JunwenZhai. A Review for the Breeding of Orchids: Current Achievements and Prospects. *Horticultural Plant Journal*2021, 7(5):380–92. doi: 10.1016/j.hpj.2021.02.006.

[4] GuoWoei-Jiun, Yu-Zu Lin, and Nean Lee. Photosynthetic Light Requirements and Effects of Low Irradiance and Daylength on PhalaenopsisAmabilis. *American Society for Horticultural Science*2012, 137(6): 465-472

[5] R. Pätz.*Biotechnology in Agriculture and Forestry 2. Crops I.*(Ed.) Y.P.S. BAJAJ/Springer-Verlag Berlin/Heidelberg/New York/Tokyo 1986, 144 Abb., 608 S., 348 DM

[6] So-Young Park, Hosakatte N. Murthy, and Kee-YoeupPaek. 2002. Rapid Propagation of Phalaenopsis from Floral Stalk-Derived Leaves. *In Vitro Cellular and Developmental Biology - Plant* 38(2):168–72. doi: 10.1079/IVP2001274

[7] SuphatRitirat, KanchitThammasiri, and SompongTe-Chato. Effect of Media and Sucrose Concentrations with or without Activated Charcoal on the Plantlet Growth of P. Cornu-Cervi (Breda) Blume andRchb. f.*Journal of Agricultural Technology* 2012 Vol. 8(6): 2077-2087

[8] PavallekoodiGnasekaran. 2012. Effects of Complex Organic Additives on Improving the Growth of PLBs of Vanda Kasem's Delight Asparagus Sp. *Australian Journal of Crop Science* 2011, 6(8): 1245-1248

[9] Kanchanapoom, K., Anuphan, T. and Pansiri, S. Effects of Total Nitrogen and BA on In Vitro Culture of Phalaenopsis.*ActaHorticulturae* 2014, 1025: 243-245

[10] RosmahMurdad, Mariam AbdLatip, Zaleha Aziz and RimiRipin. Effects of Carbon Source and Potato Homogenate on in Vitro Growth and Development of Sabah's Endangered Orchid: PhalaenopsisGigantea. *Asia-Pacific Journal of Molecular Biology and Biotechnology* 2010, 18(1):197-200

[11] Murashige, T.and Skoog, F. A Revised Medium for Rapid Growth and Bio Agsays with Tobacco Tissue Cultures. *PhysiologiaPlantarum* 1962, (15): 473-497.doi:10.1111/j.1399-3054.1962.tb08052.x

[12] Vacin, E and Went, F. Some pH changes in nutrient solution. *Bot Gardens Conserv News,* 1949,(110): 605-613

[13] Morel, G. M. Clonal multiplication of orchids. In: Withner CL (ed.), *The Orchids:Scientific Studies*, Wiley, NY, 1974

[14] Jayarama Reddy. Nutrients media used for micropropagation of orchids: A research review. *World Journal of Pharmaceutical Research*. 2019, 5(9): 1719-1732

[15] Takuya Tetsumura, Yasuyo Matsumoto, Makiko Sato, ChitoseHonsho, Kesuke Yamashita, Haruki Komatsu, Yasuhiro Sugimoto, HisatoKunitake. Evaluation of basal media for micropropagation of four highbush blueberry cultivars. *ScientiaHorticulturae*, 2008 119(1): 72-74

[16] Aktar, S., Nasiruddin, K. M. and Hossain, K. 2008. Effects of Different Media and Organic Additives Interaction on In Vitro Regeneration of Dendrobium Orchid. *J Agric Rural Dev* 6(2):69–74.

[17] Kaur, S. andBhutani, K.K. Organic growth supplement stimulants for in vitro multiplication of Cymbidium pendulum (Roxb.) Sw. *Horticultural Science (Prague)* 2012, 39(1): 47-52

[18] Huh, Yoon Sun, Joung Kwan Lee, Sang Young Nam, KeeYoeupPaek, and Gang Uk Suh. 2016. Improvement of Asymbiotic Seed Germination and Seedling Development of Cypripedium Macranthos Sw. with Organic Additives. *Journal of Plant Biotechnology* 43(1):138-145. doi: 10.5010/JPB.2016.43.1.138

[19] Kongbangkerd, Anupan, Santi Watthana, and KanokOrnSrimuang. Influence of Organic Supplements on Growth and Development of In Vitro Shoots of BulbophyllumDhaninivatiiSeidenf. *Applied Mechanics and Materials*2016, 855:42–46. doi: 10.4028/www.scientific.net/amm.855.42

[20] MaoMinea, ChitrapanPiluek, AlisaraMenakanit, and SureeyaTantiwiwat. A Study on Seed Germination and Seedling Development of Spathoglottis Bl. Orchids.*Kasetsart J. (Nat. Sci.)*2004, 38: 141-156

[21] PavallekoodiGnasekaran, Xavier Rathinam, Uma Rani Sinniah, and SreeramananSubramaniam. A Study on the Use of Organic Additives on the Protocormlike Bodies (PLBs) Growth of PhalaenopsisViolacea Orchid. *The Journal of Phytology* 2010, (1):29-33.

[22] Thimann, K. V. Auxins and the Inhibition of Plant Growth. *Biological Reviews*, 1939, 14(3): 314–337. https://doi.org/10.1111/j.1469185X.1939.tb00937.x

[23] Jean W. H. Yong, Liya Ge, Yan Fei Ng, and SweeNgin Tan. The Chemical Composition and Biological Properties of Coconut (Cocos Nucifera L.) Water. *Molecules*2009, 14(12):5144–64

[24] Duong TanNhut, Nguyen Ngoc Thi, Bui Le ThanhKhiet, and Vu Quoc Luan. Peptone Stimulates in Vitro Shoot and Root Regeneration of Avocado (Persea Americana Mill.). *ScientiaHorticulturae*2008, 115(2):124–28. doi: 10.1016/j.scienta.2007.08.011

[25] ParisaShekarriz, Mohsen Kafi ,ShirinDianatiDeilamy , MasoudMirmasoumi. Coconut Water and Peptone Improve Seed Germination and Protocorm Like Body Formation of Hybrid Phalaenopsis. *Agric. Sci. Dev.,*2014, 3(10): 317-322

[26] Philip J.Kauth, Wagner A. Vendrame, and Michael E. Kane. 2006. In Vitro Seed Culture and Seedling Development of CalopogonTuberosus. *Plant Cell, Tissue and Organ Culture,* 2006, 85(1):91-102. doi: 10.1007/s11240-005-9055-1.

[27] Wu, K.; Zeng, S.; Lin, D.; Teixeira da Silva, J.A.; Bu, Z.; Zhang, J.; Duan, J. In vitro propagation and reintroduction of the endangered Renantheraimschootiana Rolfe. *PLoS ONE* 2014, 9, e110033

[28] Moraes, M.C.; Camolesi, M.R.; Palmieri, D.A.; Bertão, M.R. Commercial fertilizers and organic additives in orchid micropropagation. *Plant Cell Cult. Micropropagation*. 2020, 16, e162.