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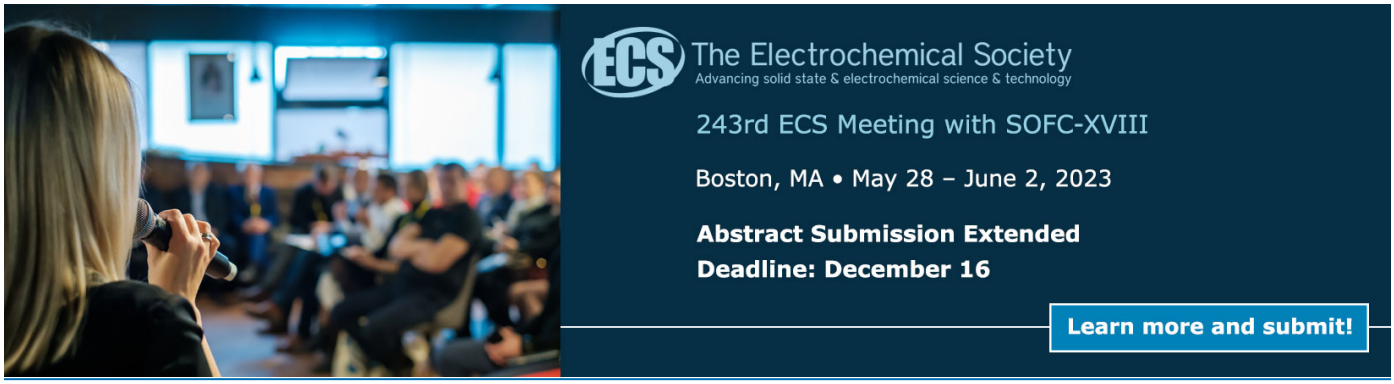

Effect of Coconut Water and Peptone in Micropropagation of *Phalaenopsis amabilis* (L.) Blume Orchid

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Abstract. *Phalaenopsis amabilis* (L.) Blume Orchid is one of the species in *Phalaenopsis* genus and are well-known as exotic and beautiful white flowers. Besides that, there is little continuity in the mass propagation of *Phalaenopsis amabilis* (L.) that produce many standardized seedlings in a relatively short period to satisfy market demand. This study was focusing on evaluating the effect of natural additives as plant growth regulators (PGR). PGR was used as nutritional constituents in the media culture to enhance the growth of explants. The additives used in this study were coconut water and peptone at different concentration, which were 5 %, 7.5 %, and 10 % of coconut water (CW) and 1 % and 2 % of peptone that supplemented in Murashige and Skoog (MS) medium. These additives were added to examine the height of plantlets, number of leaves, and number of roots after 2 months cultured. Result showed that addition of 5% CW and 2 % peptone in treatment 10 recorded the highest height of plantlet which was 1.14±0.060 cm. The highest number of leaves with 2.18±0.507 was observed in treatment 11 (7.5 % of CW and 2 % of peptone) and number of roots with 1.928±0.286 was from treatment 9 (0% of CW and 2% of peptone). Coconut water has been proved can stimulate and enhance growth and proliferation of *Phalaenopsis amabilis* (L.) plus the existence of peptone may help in development and increase the percentage of survival rate of *Phalaenopsis amabilis* (L.) due to the content of peptone which are carbon and nitrogen sources. The observation from all the treatments produces healthy explants as the natural additive can substitute the use of synthetic PGR.

1. Introduction

Orchid is one of the most diverse flowering plant family with 25000 species and 8000 genera [1]. Orchids are grown as ornamentals for their exotic beauty, their exquisite flowers and long floral life and they are valued as fascinating and highly famous plants [1]. These plants exhibit great variety in floral shape, scale, colour, fragrance, and texture. Commercial manufacturing has grown substantially and has become a market that is very profitable. Many species cultivated for the beauty of their flowers such as *Cymbidium, Paphiopedilum,* and *Phalaenopsis* [1].

P. amabilis (L.) is an important national flower of Indonesia as a parent for orchid breeding, so it needs a good strategy for growing high numbers of plants. The name of the genus derived from the Greek phalaino (moth) and opsis (appearance), referring to some species of moth-like flowers [2]. The moth orchid is one of the orchids identified from the Far East as the hybrids of these orchids are famous as the houseplant. In general, *Phalaenopsis* flower characters are one of the essential elements that attract considerable attention from orchid breeders and growers [2].

Phalaenopsis has a monopodial growth pattern. It rarely displays branching and rarely initiates fresh secondary shoots, as the flower branch is usually formed from each leaf's axial bud. The vegetative propagation of this type of orchid (or asexual propagation) is difficult and slows growth naturally [3]. Naturally, this orchid is propagated by seed, which naturally grows on the stalk of the flower. Frequent research on the development of Phalaenopsis has shown that it can be propagated by cultivating the seeds, by cultivating the nodes on the inflorescence, by in vitro cultivating the leaves that emerge from those nodes, by cultivating protocorm-like bodies (PLBs), and by seed-based plantlets [1].

In the article by Michael (2012), the use of coconut water as a nutrient supplement with the optimum concentration shows a positive result in improving the shoot and node growth of the explants. Besides, peptones are plant or animal tissue enzymatically prepared under rigidly defined processing conditions. Therefore, this study will investigate the impacts of the various concentrations on coconut water as an organic addictive and two different concentrations of peptone, which will be replacing the plant growth regulators (PGRs) that tend to be used in the media preparation. Furthermore, for this research, the application of coconut water and peptone was first applied to *Phalaenopsis amabilis* (L.) Blume Orchid.

2. Material and Method

2.1 Plant material

The *P. amabilis* (L.) Blume Orchid was gained from Tissue Culture Laboratory of Universiti Malaysia Kelantan as the explant of *Phalaenopsis amabilis* (L.) Blume Orchid was successfully cultured and maintained in the laboratory.

2.2 Additive source

The coconut water fruit (*Cocos nucifera* L.) was bought from the local market in Jeli while peptone was obtained from the Tissue Culture Laboratory of Universiti Malaysia Kelantan.

2.3 Preparation of coconut water

Coconut water was sieved 2 to 3 times through a sterile double-folded muslin filter cloth to eliminate the unnecessary object. The coconut water was stored at 4 °C in the fridge. The MS medium applied with different concentrations of coconut water, which were 10 ml of 5 %, 15 ml of 7.5 %, and 20 ml of 10 % in 200 ml of MS media.

2.4 Media preparation

The MS medium used as the base medium augmented by varying amounts of coconut water (0 ml of 0 %, 10 ml of 5 %, 15 ml of 7.5 % and 20 ml of 10 %) while peptone used was 0.2 g of 1 % and 0.4 g of 2 %. Table 3.2 showed the division for the coconut water and peptone in 12 treatments. After media preparation, the pH of the media was balanced within the range of 5.8 by using pH meter and be regulated with NaOH and HCl [4]. The media was autoclaved at 121˚C for 20 minutes.

Table 1. The treatment of MS media with different concentration of coconut water and peptone.

Coconut water (%) / Peptone (%)	0 %	5 %	7.5 %	10 %
0 %	T_1	T_2	T_3	T_4
1 %	T_5	T_6	T_7	T_8
2 %	T_9	T_{10}	T_{11}	T_{12}

* Treatment (T)

2.5 Culture initiation of plant

The healthy plantlets were used for subculture into the jars. It was cut about 1 cm – 2 cm long to get uniformity under controlled conditions. Then, the plantlets were transferred into the jars that contained a different concentration of additives, which were coconut water and peptone. Each of

the treatments consisted of 5 jars, and every jar contained 3 plantlets. Lastly, the culture of *P. amabilis* (L.) was placed in a culture room at 24.2 °C for 16 h photoperiods as for the growth and multiplication rate observed for 2 months.

2.6 Experimental design and statistical analysis

This study was settled down in a Completely Randomized Design (CRD). Within 2 months, the height of plantlets, number of leaves and number of roots was observed and collected. The mean value of the 12 treatments were analyzed by using one – way ANOVA using SPSS ver. 20 software. The different mean number between every treatment were compared by using Duncan's multiple range test within the range of P value ≤ 0.05 [5].

3. Result and Discussion

3.1 Effect of different concentration of coconut water and peptone on the height of Phalaenopsis amabilis (L.) Blume orchids

The effects of different concentrations of coconut water and peptone on the height of *P. amabilis* (L.) were shown in Figure 1, where the result was shown after 2 months of monitoring. All the treatments showed a significant value within the group, which was 0.000 (p = 0.000), which means smaller and below 0.05. T10 has the highest number of plantlets in 2 months, followed by T2, T11, and T8. In contrast, the rest of the treatments show the increment growth height of plantlets but not in the highest range compared to the control (T1).

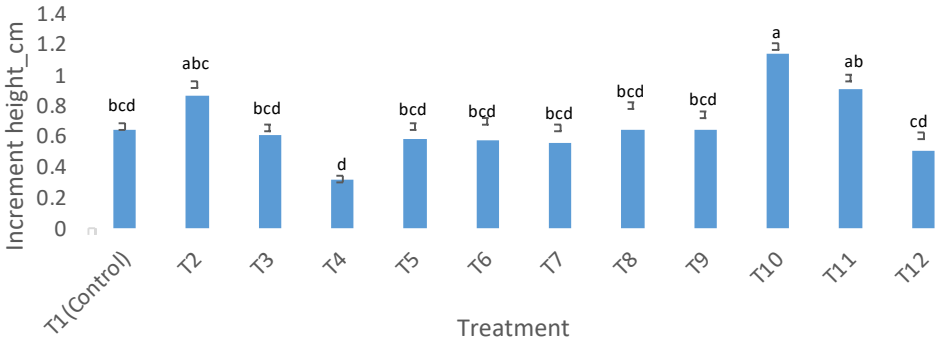


Figure 1. The effect of different concentration of coconut water and peptone on height of *Phalaenopsis amabilis* (L.) Blume Orchids. Common letters within the column indicate non-significant different between mean using Duncan's multiple range test (p ≤ 0.05).

The best treatment that shows the highest increment growth height of *P. amabilis* (L.) was shown in Figure 1. According to Winarto and Silva [6], coconut water has been used as an organic additive since the early 1940s and has proved to be effective in stimulating and enhancing the growth and proliferation of many types of orchids, including *P. amabilis* (L.). Besides that, the peptone also helps enhance the growth of the explants as the peptone itself contains carbon and nitrogen sources for the in vitro culture of plants. That may help in development and effectively increase the percentage survival rate for *P. amabilis* (L.) as one of the terrestrial orchids [7].

Natural additives like coconut water can enhance the growth of the explants because of the high content of nutrition and hormonal substances like diphenyl urea in the coconut water like diphenyl urea plays the role of cytokinin and auxin that help in inducing the growth of explants [8]. While peptones play a crucial role in the activation of some genes, the peptone content also has chlorophyll in the photosynthesis process [7].

As for this study, the different concentrations of coconut water and peptone for all the treatments of *P. amabilis* (L.) have an effect on the growth of them. As T10 and T11 increased in height, the growth of *P. amabilis* (L.) showed a positive reaction to the peptone as the highest concentration of peptone was added in the MS medium of both treatments based on Figure 2.

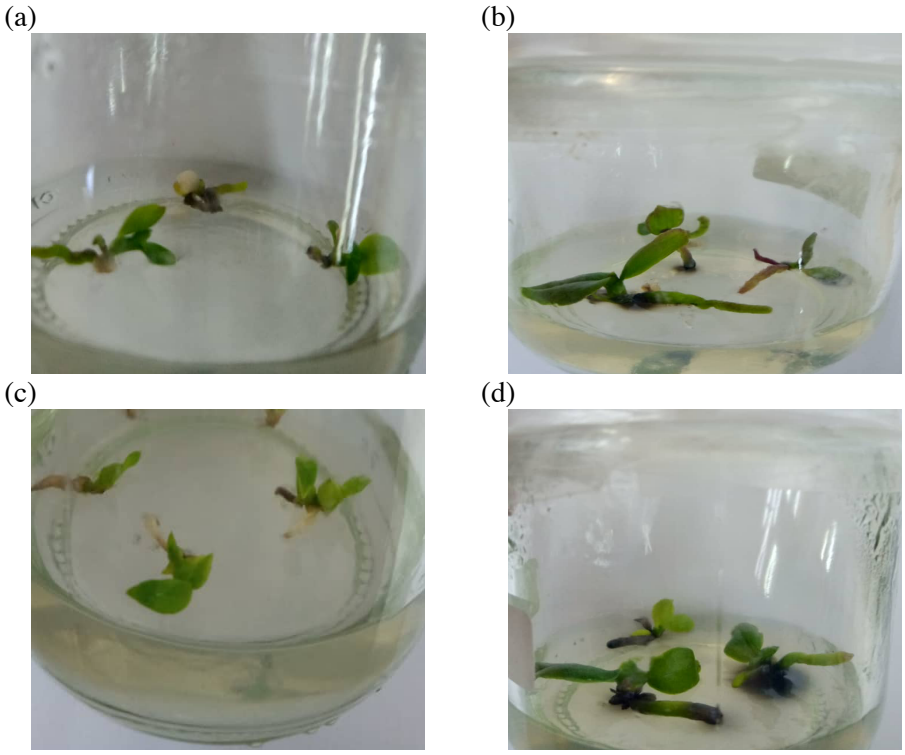


Figure 2. The height of *Phalaenopsis amabilis* (L.) Blume orchid plantlets under 2 best treatments: (a) T10 before 2 months, (b) T10 after 2 months, (c) T11 before 2 months, and (d) T11 after 2 months.

3.2 Effect of different concentration of coconut water and peptone on the number of leaves of P. amabilis (L.) Blume orchids

The relationship between the effects of different concentrations of coconut water and peptone on the number of leaves of *P. amabilis* (L.) was shown in Figure 3, where the result is shown after 2 months of observation. All the treatments show a significant mean value between the groups, which was 0.318 (higher than $p \leq 0.05$). T11 had the highest mean number of leaves in 2 months, which the increment was 2.18. While the rest of the treatments show mean values for the number of leaves but not the highest range compared to the control (T1).

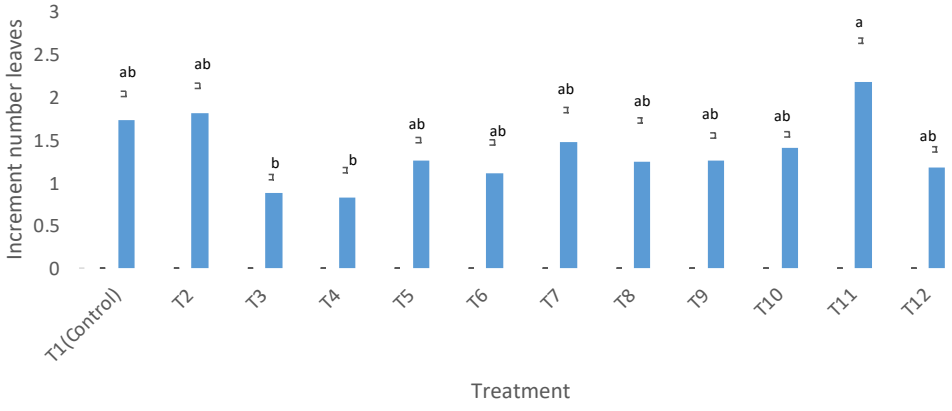


Figure 3. The effect of different concentration of coconut water and peptone on number of leaves of *Phalaenopsis amabilis* (L.) Blume Orchid. Common letters within the column indicate non-significant different between mean using Duncan's multiple range test ($p \leq 0.05$).

Based on Figure 3, the different concentrations of coconut water could give different growth patterns in enhancing the number of leaves of *P. amabilis* (L.). As the highest mean values in the number of leaves, the appropriate concentrations of coconut water and peptone in the multiplication of the number of leaves were 7.5% and 2% of peptone, respectively. As for the other treatments, they show a significant difference compared to control (T1).

According to a previous study by Shen et al. [11], basically in plant tissue culture, the phenolic compounds in the media usually inhibit the growth of the plantlet and the proliferation of leaves. The wounded cells' contents became mixed when the explants were excised and the phenolic compounds were oxidized, resulting in toxic secretions into the culture medium and, eventually, the cultures' necrotic browning. The amounts of phenolic compounds, such as flavanols, in cells are determined by light and other factors [12]. Light-induced extreme browning and necrosis of the explants completely retarded direct somatic embryogenesis in the *Phalaenopsis* leaf cultures.

The supplementation of different concentrations of additives like coconut water and peptone had a profound effect on the growth of *P. amabilis* (L.) based on figure 4 in terms of the number of leaves. Sugars, amino acids, vitamins, enzymes, and organic acids were found in coconut water, which influenced the growth of *P. amabilis* (L.) Blume Orchid leaves. According to Utami and Hariyanto [13], the treatment that supplements with peptone shows a positive result in the multiplication of shoots and leaves since it contains high amounts of vitamins and amino acids, including thiamine, biotin, nitrogen, and pyridoxine.

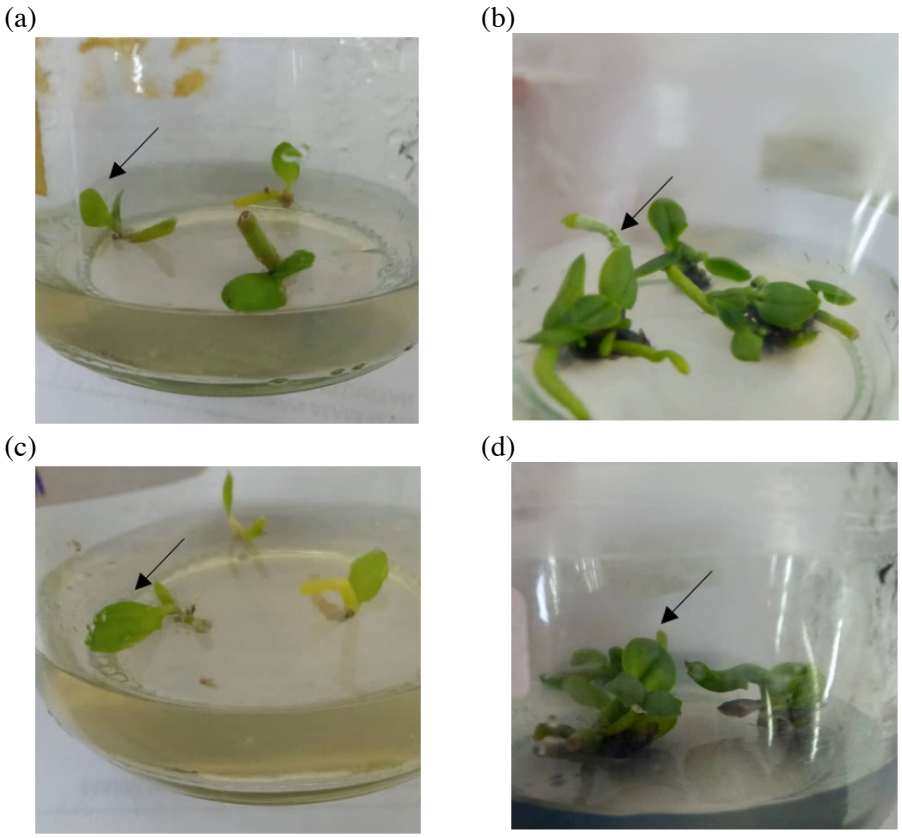


Figure 4. The number of leaves of *Phalaenopsis amabilis* (L.) Blume Orchid plantlets: (a) T11 before 2 months, (b) T 11 after 2 months, (c) T2 before 2 months, and (d) T2 after 2 months.

3.3 Effect of different concentration of coconut water and peptone on the number of roots of P. amabilis (L.) blume orchids

The relationship between the effect of different concentrations of coconut water and peptone on the number of roots of *P. amabilis* (L.) was shown in Figure 5 below, where the result is shown after 2 months of observation. The significant value between the mean group was 0.202 (higher

than $p \leq 0.05$). T9 shows the greatest increment in 2 months compared to others. The rest of the treatments did not really enhance the growth of roots compared to the control treatment (T1).

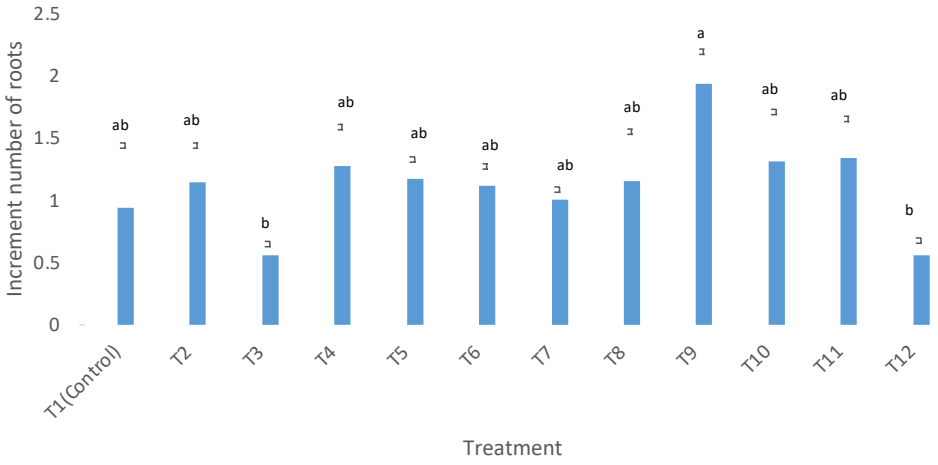


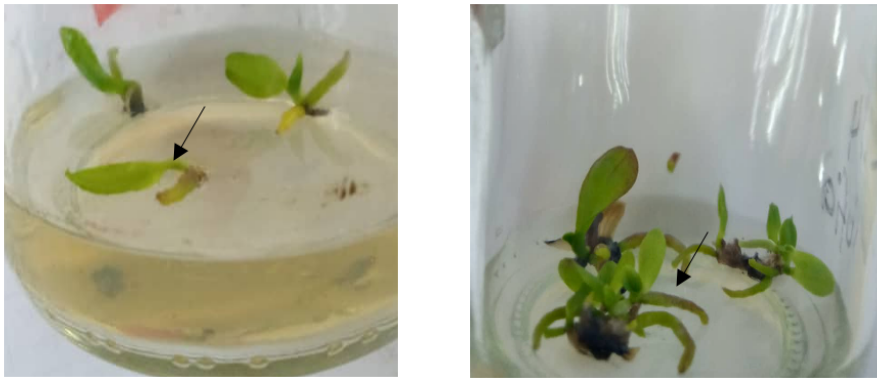
Figure 5. The effect of different concentration of coconut water and peptone on number of roots of *P. amabilis* (L.). Common letters within the column indicate non-significant different between mean using Duncan's multiple range test ($p \leq 0.05$)

According to a previous study by Zanello and Cardoso [15], as the green root tips quickly turned yellow and brown. The root cap later cracked, and the growth of the tumour protruded from the root. The cancer is protected by a multiple cell layered root cap and gradual death of the outermost layers gives the tumour surface a rough, whitish appearance that usually occurs in some different types of orchids.

The root growth of *P. amabilis* (L.) did not show a positive reaction to the presence of coconut water as the additional additive in the MS medium in terms of numbers of roots or induction of roots. Recent studies have shown that coconut water in the culture medium significantly stimulates the formation of roots in some orchid species [6]. Organic additives such as coconut water may have a positive impact as the culture grows and requires optimization and adjustment. Nevertheless, some organic additives could react differently between the plants as some show positive results as they enhanced the growth, but some show negative results as they inhibited the growth of the plant [15]. The use of coconut water in the *vitro* culture of *P. amabilis* (L.) did not give positive results towards the growth and formation of roots.

However, in this research, the most efficient culture medium was that supplemented with peptone in T9, which had the highest growth in 2 months based on Figure 6. Organic growth adjunct supplementation in the medium of orchid culture is a simple, realistic, beneficial, and traditional method for improving media used for commercial production.

(a) (b)



(c) (d)

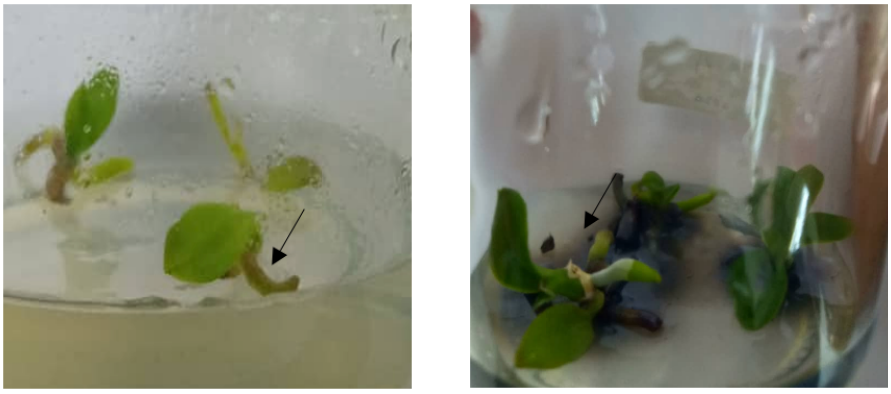


Figure 6. The number of roots of *Phalaenopsis amabilis* (L.) Blume Orchid plantlets: (a) T9 before 2 months, (b) T9 after 2 months, (c) T10 before 2 months, and (d) T10 after 2 months.

4. Conclusion

From the experiment, the development and growth of an *in vitro* culture of *P. amabilis* (L.) that was run by using different concentrations of coconut water and peptone was successfully developed. The height of plantlets of *P. amabilis* (L.), the MS medium that contained 5% of coconut water and 2% of peptone was proved to be the best treatment for the regeneration of the plantlet's height. Meanwhile, 7.5% of coconut water with 2% peptone was the best treatment for leaves number and root development was the best with 2% peptone.

Acknowledgments

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