Effect of selected substrates and chitosan on growth performance of orchid tissue culture seedling under net house

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Abstract. Orchids are considered as the most beautiful and significant cut blossom and pruned plants throughout the world. Therefore tissue culture technique was used as one of the alternative to mass propagate the orchids to fulfil the demand. This study was conducted to find out the effect of chitosan and different substrates on growth performance of Dendrobium Shavin White orchid seedling from tissue culture environment to net house. The tissue cultured plants went through prehardening stage to reduce mortality after the switch to ex vitro conditions. Five (5) treatments consist of combination substrates were tested which were T1: rice husk + chitosan, T2: peat moss + chitosan, T3: EFB + chitosan, T4: rice husk + peat moss + chitosan and T5: rice husk + peat moss + EFB + chitosan whereas charcoal as a control (T0).

The result showed that the highest plant height and leaf length which did not significantly different were T3 (6.20cm plant height, 4.83cm leaf length), and T5 (6.55cm plant height, 4.51cm leaf length). Meanwhile, T3 was an effective for number of leaf production with 4.14 leaf count. Hence, this data will be used to acclimatize tissue cultured of Dendrobium Shavin White orchid for commercial used.

1. Introduction

Most of nature lovers and scientists were fascinated with Orchidaceae more than other plants group. The reason Orchidaceae get the attention was due to their model example of coevolution, their function, great structural and the interaction made between other organisms [1]. Dendrobium was known as the second largest genus of Orchidaceae. There were more than 27,800 of natural orchid species and 100,000 of hybrids orchid [2]. Orchids may be particularly vulnerable to over-harvest because many species have a limited range and/or occur at low densities due to a variety of interacting factors such as the restricted distribution of mycorrhizal symbionts, specialized pollination mechanisms, recent speciation, and habitat specificity [3]. By doing tissue culture especially on Dendrobium it could help to produce the orchids in large numbers with more quality or resistance to diseases in limited time. Tissue culture technique was used massively to produce plants free from disease under in vitro conditions. However, the problem facing by planters is the survivability of the tissue cultured plant to adapt to the real environment that different from laboratory surrounding. Mostly there were damages occurred during the transfer from in vitro to ex vitro condition [4]. Before
getting into the environment, tissue cultured plants required hardening treatment to prevent high mortality after the switch to ex-vitro conditions [5]. Plantlets at these levels are exposed to high percentages and damages [6]. Therefore, the requirement to get a high survival rate of in vitro plantlet on ex vitro condition by choose effective growth medium in acclimatization stages [7]. Hardening treatment for tissue cultured explants, the situation for the growth of explants, for example, the mixture of potting media, light condition and humidity of net house, also the genotype are playing important roles for the success of acclimatization of Dendrobium plants [7]. Plant hardening can begin when plants were still in vitro conditions, contributed to better survival percentages at the acclimatization stage in greenhouse environment [7]. In the wild, orchids exposed their roots to absorb moisture from dew and as well as from humid air [8]. Thereby, to grow orchids in artificial states, substrate with good capillary action, aeration, nutrient and water holding capacities should be considered [9, 10].

An effective potting media will supply a good ambient effect for better drainage, enough moisture retention and great aeration, which were vital to orchid’s growth [11, 12]. Dematte and Graziano [13] tested various media on Dendrobium Nobile such as mix of coconut bark, Eucalyptus bark, and charcoal, fern fiber tree, Eucalyptus grandis bark and pressed coconut bark. Peat moss has long been a recommended ingredient of terrestrial mixes [14]. Peat moss has a high water retention capacity and it does stay relatively intact for several years [15]. High survival (95%) of Dendrobium nobile plantlets was observed when they were transferred to small plastic pots containing peat moss, wood charcoal, and brick pieces (1:1:1) [16]. Rice husk is selected for use as a pioneering material due to its granular structure, infertility in water; chemical stability; high mechanical strength and local availability are virtually no cost [17]. These waste was consider as a great planting media because of their physical properties, chemical reaction and light weight [18]. There was a research on rice husk with the test of mix between vermiculite and carbonized rice husk resulted with the development of D. nobile plantlets for 180 days after transplanted [19, 20]. Oil palm empty fruit bunch (EFB) is a waste from oil palm industry and have high potential to recycle and use as energy sources and agriculture input. It contain high cellulose(43-65%) and lignin (13-25%) content and is proven raw materials for biocomposite and mulching purposes [21]. Chitosan is a biopolymer, a chitin derivative, a compound that is safe for the environment. This compound is characterized by unique properties, such as bioactivities and biocompatibility [22]. Plants treated with chitosan may be less susceptible to stress caused by high or low temperature, poor conditions, drought, and salinity [23]. Acclimatization of Dendrobium Shavin White orchid plantlet from tissue culture was held under the net house to make sure it can adapt to the environment. This orchid needed a good substrate as a media for the better growth of plant ex vitro. Therefore, the objective of this study was to determine the effect of chitosan and different substrates on growth performance of transplanted Dendrobium Shavin White orchid from tissue culture environment to net house.

2. Material and Methods

2.1. Plant materials
The plant materials used in this study were in vitro plantlets of Dendrobium Shavin White orchid which was provided from the Plant Tissue Culture Laboratory at Universiti Malaysia Kelantan, Jeli Campus. The selected plantlets were more than 6 cm height, have developed roots and the leaves were more than three.

2.2. Substrate preparation
The substrate used for acclimatization were prepared and placed in the plastic pots. The substrates used were peat moss, rice husk and oil palm empty fruit bunch (EFB). The control media for this study was charcoal. The substrates were autoclaved to rid of any unwanted microorganism and any unwanted seeds before the media preparation. While for the charcoal, it was soaked in diluted fungicide to rid of any unwanted fungus and prevent from plantlets infected with fungus. 2 ml of 15ppm chitosan was sprayed every two weeks. There were five (5) treatments involved in this study
that were $\text{T1}$: rice husk + chitosan, $\text{T2}$: peat moss + chitosan, $\text{T3}$: EFB + chitosan, $\text{T4}$: rice husk + peat moss + chitosan and $\text{T5}$: rice husk + peat moss + EFB + chitosan whereas charcoal as a control (T0) substrate. The treatments of the media used were in the ratio of mixed substrates as in Table 1.

| Code | Treatment                                      | Substrate Ratio |
|------|------------------------------------------------|-----------------|
| T0   | Charcoal (Control)                             | 1               |
| T1   | Rice Husk + Chitosan                           | 1               |
| T2   | Peat Moss + Chitosan                           | 1               |
| T3   | Oil Palm EFB + Chitosan                        | 1               |
| T4   | Rice Husk + Peat Moss + Chitosan               | 3:2             |
| T5   | Rice Husk + Peat Moss + Oil Palm EFB + Chitosan| 3:2:2           |

2.3. Data analysis

The pots were transferred at the experimental plot under the net house by using complete randomized design (CRD). The samples were in six (6) replication. The data were collected based on the height of the *Dendrobium* Shavin White, the length of the leaf and the number of the leaf every 2 weeks interval until 12 weeks. The data were analyzed by using Statistical Package for Social Sciences software (SPSS) through One-way ANOVA and the Duncan test used to find the variance from the whole set of data.

3. Results and Discussion

Based on Figure 1A, the highest plant height was in $\text{T5}$ (6.55cm) which was combination of rice husk, peat moss, oil palm EFB and chitosan followed by $\text{T3}$ (6.20cm) which was a mixture of oil palm EFB with chitosan. This $\text{T5}$ and $\text{T3}$ treatment were not significantly different and surpassed other treatments including control (T0). $\text{T1}$ (rice husk and chitosan), $\text{T2}$ (peat moss and chitosan) and $\text{T4}$ (rice husk, peat moss and chitosan) showed lower height compared to $\text{T5}$ and $\text{T3}$ treatment with height of 4.59cm, 4.56cm and 3.71cm respectively. The outcome of the study exhibited that the substrates had high water capacity and aeration in the medium was suitable to use in acclimatization of *Dendrobium* Shavin White plantlets. This could be the main reason of $\text{T5}$ and $\text{T3}$ treatments showed good performance between other treatments. Rice husk and peat moss were good water holding capacity and provide nutrients to plants while oil palm EFB was good for aeration due to fibrous materials [24, 25]. Jimenez et al. [26] reported that a mixture of peat moss with perlite allows a certain degree of water retention and permits good drainage and aeration of roots, thus suitable for this orchid to grow optimum and showed high growth. This result indicated that addition of chitosan did improve the height of orchid seedling. Similarly, Zakaria et al. [27] mentioned that addition of chitosan did improved the acclimatization of *Solanum tuberosum* L. in the green house.
Figure 1. The effect of different substrate on growth performance of *Dendrobium* Shavin White orchid. 
A- Plant height, B- Leaf length, C- Leaf number. T0; Control, T1; Rice husk + Chitosan, T2; Peat moss + Chitosan, T3; Oil palm EFB + Chitosan, T4; Rice husk + Peat moss + Chitosan, T5; Rice husk + Peat moss + Oil palm EFB + Chitosan. Results with the same letter are not statistically different (one-way ANOVA, Duncan test, p<0.05).
Figure 2. The growth of Dendrobium Shavin White orchid seedling in different substrate. A-T1; Rice husk + Chitosan, B-T3; Oil palm EFB + Chitosan, C-T4; Rice husk + Peat moss + Chitosan, D-T5; Rice husk + Peat moss + Oil palm EFB + Chitosan. Bar=1cm

The results in Figure 2 showed the leaf length between treatments. Same as height of the plant, the best result for length of leaf were T3 (4.83cm) and T5 (4.51cm) treatments which were not significantly different between each other. The results were followed by treatment of T2 (3.60cm), T1 (3.23cm), T4 (2.58cm) and lastly T0 (2.22cm). Oil palm EFB can be a good alternative for orchid propagation because it was fibrous material which can supply enough aeration and space for root development that help in plant development. It was proved by Qian et al. [11] that reported an effective media such as peat moss and oil palm EFB give good effect for better drainage, moisture retention and great aeration which were important to orchid growth. Addition of chitosan could improve the growth performance of this orchid. Barka and Eullaffroy [28] also reported that chitogel
solution enhanced the roots and biomass shoots, photosynthesis and parameters related to grapevine plants in vitro culture. The application of sprays chitosan directly on young orchid plants enhances the plant growth [29]. It is because chitosan have an ability to stimulate the induction and the differentiation of Dendrobium Phalaenopsis orchid plant [30] by exhibits several reactive amino side groups which enhance the applicability of chitosan and offer the possibility of formation of a large variety of chitosan derivatives [22].

Among all the treatments used in this study, the highest leaf number was observed in the T3 treatments (Figure 1C) with the mean value of 4.14. T3 treatment consist of oil palm EFB and chitosan. It followed by T1, T5, and T2 treatments which were not significantly different with leaf number range from 3.28 to 3.88. The lowest leaf number was observed in T4 treatment with 2.3 leaf per plantlet. It was proved that oil palm EFB was effective to use as a substrate for acclimatization of Dendrobium Shavin White orchid in all the parameters tested. Oil palm EFB have been used as mulching to oil palm tree which able to increase the yield and also improve nitrogen and potassium availability in the soil [31]. All the fibre based media provided vigorous plant growth and sufficient nutrient supplies of P and Mg, but required additional N and K nutrients. Hoe [31] reported that banana tissue culture seedling were successfully acclimatized using oil palm waste including EFB compared to soil alone. Thus, substrate generally represent by the blending of two or more materials to optimize usage of condition as it is difficult for single substrate to provide all the necessary characteristics for optimum plant development [32]. Rice husk and peat moss were not suitable to combine together as this combination of substrate showed the lowest growth performance for plant height, leaf length and leaf number. Chitosan was believed to promote the growth of Dendrobium Shavin White seedling as it function is to improve the formation of plant roots under drought pressure, increase the chlorophyll content, and encourage the number of nodes [33]. The Dendrobium Shavin White seedling adaptation to environment were improve significantly from their growth development results (Figure 2). According to Pospisilova et al. [5], the growths of acclimatized Spathiphyllum floribundum were based on adaptation stages with start from slow growth followed by fast growth.

4. Conclusion

Acclimatization of Dendrobium Shavin White tissue culture seedlings under net house were successfully improve orchid growth. Combination of rice husk, peat moss, oil palm EFB and chitosan did improve plant height and leaf length of this orchid while oil palm EFB and chitosan media was suitable in producing number of leaf. Hence, this data will be used to enhance the growth performance in acclimatization stage for tissue culture of Dendrobium Shavin White orchid for commercial used.

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