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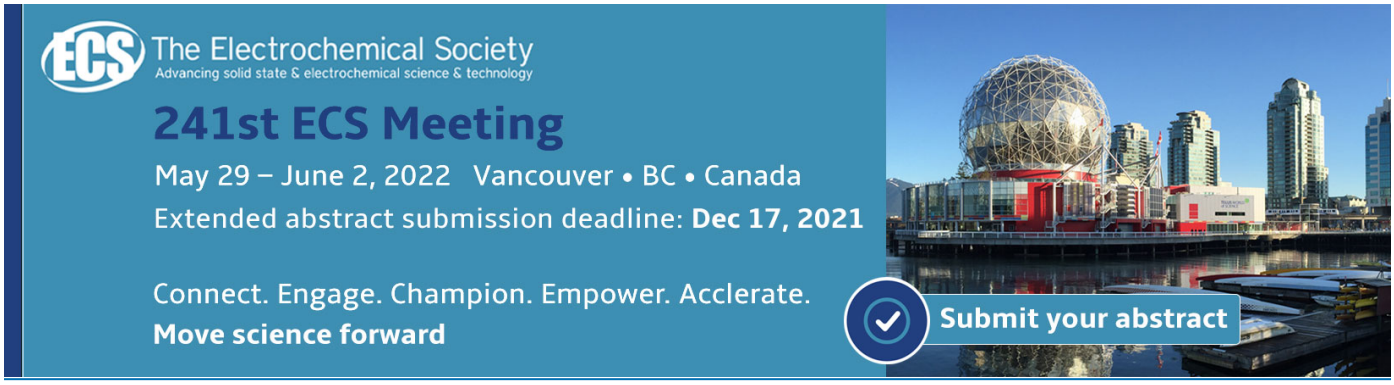

Effect of selected substrates and chitosan on growth performance of orchid tissue culture seedling under net house

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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 labarotory 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 **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)** substrate. The treatments of the media used were in the ratio of mixed substrates as in Table 1.

Table 1. Ratio of substrates in the treatments.

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 transfered 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 **T5** (6.55cm) which was combination of rice husk, peat moss, oil palm EFB and chitosan followed by **T3** (6.20cm) which was a mixture of oil palm EFB with chitosan. This **T5** and **T3** treatment were not significantly different and surpassed other treatments including control (**T0**). **T1** (rice husk and chitosan), **T2** (peat moss and chitosan) and **T4** (rice husk, peat moss and chitosan) showed lower height compared to **T5** and **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 **T5** and **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 hight 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.

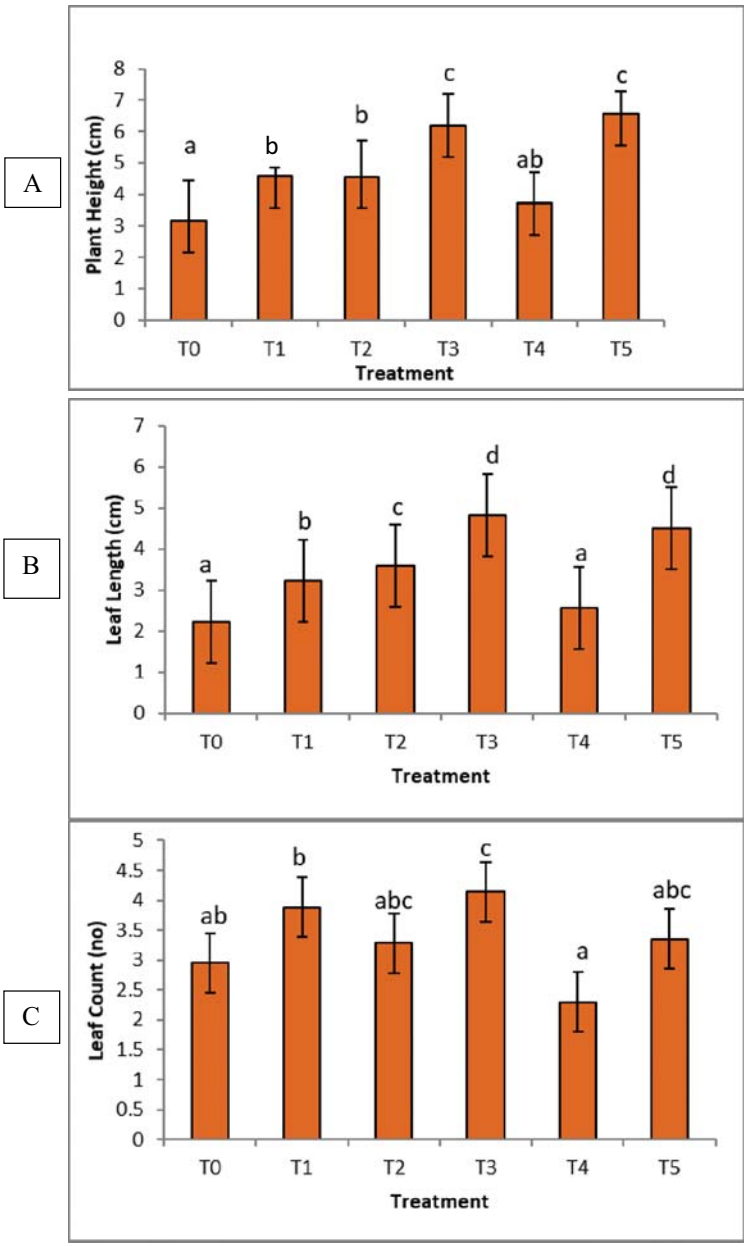

Figure 1. The effect of different substrate on growth performance of *Dendrobium* Shavin White orchid. A- Plant height, B- Leaf length, C- Leaf number. T0; Control, T1; Rice husk + Chitosan, T2; Peat moss + Chitosan, T3; Oil palm EFB + Chitosan, T4; Rice husk + Peat moss + Chitosan, T5; Rice husk + Peat 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 Posposilova 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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References

[1] Rasmussen H N, Dixon K W and Jersa J 2015 Germination and seedling establishment in orchids : a complex of requirements *Ann Bot* **116 (3)** 391–02
[2] Chase M W, Cameron K M, Freudenstein J V, Pridgeon A M, Salazar G and van den Berg C 2015 An updated classification of Orchidaceae *Bot J Linn Soc* **177** 151–74
[3] Pathak P and Rajeevan PK 2019 2 Global Orchid Industry *Creative Commons Attribution-Non Commercial-NoDerivs 3.0 License* pp 13-19
[4] Teixeira S J A, Hossain M M, Sharma M, Dobránszki J, Cardoso J C and Zeng S 2017 Acclimatization of in Vitro -derived Dendrobium *Hortic. Plant J.* **3**(3) 110–24
[5] Pospisilova J, Ticha I, Kadlecek P, Haisel D and Plzakova S 1999 Acclimatization of micropropagated plants to ex-vitro conditions *Biol Plant* **42** 481-97

[6] Ortega-LOeza M M, Salgado-Garciglia R,Gómez-Alonso C and Ávila-Díaz I 2011 Acclimatization of the endangered Mexican Epiphytic Orchid, Laeliaspeciosa (H.B.K.) Schltr. *Eur J Environ Sci* **1** (2) 48-54
[7] Sharma M C 2017 Acclimatization of in Vitro-derived Dendrobium *Hort Plant J* **3(3)** 110-124
[8] Fadelah A A 2009 Field performance of tissue cultured micropropagated Dendrobium orchid hybrids *Acta Hortic* **819** 499-06
[9] Spomer L A, Berry W L and Tibbitts T W 1997 Plant culture in solid media, in: Langhans, R.W., Tibbitts, T.W. (Eds.) *Plant Growth Chamber Handbook* (Iowa State University of Science and Technology, Ames) pp 105–18
[10] Di Benedetto A 2007 Alternative substrates for potted ornamental plants based on Argentinean peat and Argentinean river waste: A review *Floriculture Ornamental Biotechnol* **7** 90–101
[11] Qian W L, Zhang J X, Wu K L and Zeng S J 2013 Study on propagation and cultivation technique of *Dendrobium* huoshanense seedlings *J Tropical Subtropical Bot* **21** 240–46
[12] Xiao Y and Zhang Y Z 2013 The effect of media and plant training and season on the pre-planting of the tissue culture shoots of *Dendrobium* candidum Wall. ex Lindl *J Xinyang Agric College* **23** 93–94
[13] Demattê M E S P and Graziano T T 2000 Growth of *Dendrobium nobile* Lindl. as related with nutrient concentration in the growing media *Acta Hort* **511** 265–70
[14] Silva F F, Wallach R and Chen Y 1993 Hydraulic properties of Sphagnum peat moss and tuff (scoria) and their potential effects on water availability *Plant Soil* **154** 119–26
[15] Orchids V 2002, December 24 A Comprehensive Guide to Orchid Culture Retrieved from *Orchid USA*
[16] Nayak N R 2002 Establishment of thin cross section (TCS) culture method for rapid micropropagation of *Cymbidium aloifolium* (L.) Sw. and *Dendrobium nobile* Lindl. (Orchidaceae) *Sci. Hortic.* 107-16
[17] Awang Y and Shazmi A 2009 Chemical and Physical Characteristics of Cocopeat-Based Media Mixtures and Their Effects on the Growth and Development of Celosia cristata *Am J. Agric. Biol. Sci.* 63-71
[18] Alzrog A M, Mohamed A S and Zakaria R 2013 Effect of planting media (rice husk and coco peat) on the uptake of cadmium and some micronutrients in chilli (*Capsicum Annum* L.) *Biology* **2**(3) 76–82
[19] Duan J and Duan Y P 2013 The High Efficient CultivationTechniques of *Dendrobium* (Fujian Science and Technology Press, Fuzhou) pp 11-15
[20] Anetal Y F, Zhang Y Q, Zhou L M and Feng D Q 2014 The research progress of cultivation matrixes of *Dendrobium candidum China Pharmacy* **25** 2581–83
[21] Shinoj S, Visvanathan R, Panigrahi S and Kochubabu M 2011 Oil palm fiber (OPF) and its composites: A review *Ind Crops Prod.* **33** 7-22
[22] Dias A M A and Cortez A 2013 Development of greener multi-responsive chitosan biomaterials doped with biocompatible ammonium ionic liquids *ACS Sustainable Chem. Eng.* 1480-92
[23] Abdullah N and Sulaiman F 2013 The Oil Palm Wastes in Malaysia The Oil Palm Wastes in Malaysia *Biomass Now: Sustainable Growth and Use* (Croatia: InTech) pp 75-100
[23] Lizarraga P E G, Torres P, Moreno M E and Miranda C 2011 Chitosan application in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level *Afr. J. Biotechnol.* **10**(34) 6439-46
[25] Baharuddin A S, Wakisaka M, Shirai Y, Abd A S and Abdul Rahman N A H M A 2009 Co-composting of empty fruit bunches and partially treated palm oil mill effluents in pilot scale *Int. J. Agric. Res*. **4**(2) 69–78
[26] Jimenez V, Masis S and Eric G 2011 Effect of macronutrients and sucrose concentration on in vitro grow of *Drosera capensis* L. (Droseraceae) plants, and evaluation of six substrates for acclimatization *Propag. Ornam. Plants* 47-68

[27] Zakaria R A, Zanjani B M and Sedghi E 2009 Effect ofin vitro chitosan application on growthand minituber yield ofSolanum tuberosum L *Plant Soil Environ.* **55**(6) 252–56
[28] Barka E A, Eullaffroy P, Clement C and Vernet G 2004 Chitosan improves development,and protectsVitis viniferaL. against *Botrytis cinerea Plant Cell Rep* **22** 608–14
[29] Chandrkrachang S 2002 The applications of chitin in agriculture in Thailand *Advances in Chitin Science* **5** 458–62
[30] Nge K L, New N, Chandrkrachang and Stevens W F 2006 Chitosan as a growth stimulator in orchid tissue culture *Plant Sci.* **170** 1185-1190
[31] Hoe T K 2014 Utilization of oil palm fruits mesocarp fibres waste as growing media for banana tissue culture seedling in Malaysia J. *Adv. Agric. Technol*. **1** 52-55
[32] Silva K B, Reiniger L R S, Stefanel C M and Rabaiolli S M S 2020 Sucrose and substrates on the acclimatization of micropropagated *Luehea divaricata plants Floresta e Ambiente* **27**(1) e20171170
[33] Gornik K, Grzesik M and Duda B R 2008 The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress *J. Fruit Ornam* **16** 333-43