Effect of Various Rhythms on *In Vitro* Seed Germination of Several Orchid Species

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***Abstract*— It had been found that sound plays it own role in influencing plant growth by better development and even positive genetic characters. However, only several researches are being conducted to date on determining type of sounds that effect the plants growth. Therefore, the objective of this study is to compare the *in vitro* growth rate of 3 different types of orchids exposed to 5 genres of rhythm. The 3 species of orchid seeds were from *Bulbophyllum longisepalum*, *Dendrobium anosmum* and *Dendrobium tanii*. The types of genre used were Instrumental group, Ballad group, Yasin group, Hiphop and Rock group. Each seeds was exposed to the different rhythm for 8 hours per day for 3 months and *in vitro* cultured under 25±2°C in dark condition for 8 weeks and further cultivated under light for 4 weeks. The seeds were cultured with sterile technique on half strength Murashige and Skoog (MS) medium supplemented with 30g/L sucrose, 1 g/L activated charcoal, 2 g/L agar and 2 g/L peptone. At the end of our analysis, we found that music gave positive influence on exposed plant as compared to the non-exposed. The highest seed germination, D. *anosmum* showed at 95% growth rate when exposed to Yasin rhythm, while D. *tanii* and B. *longisepalum* showed highest growth rate when exposed to Ballad and Rock rhythm at 90%, respectively. As the result, it is assured that plants definitely showed some effects when exposed to the music. The findings from this research showed that, different plant species required different rhythm for their best growth.**

Keywords*— rhythm; seed germination; *Bulbophyllum longisepalum; Dendrobium anosmum; Dendrobium tanii

INTRODUCTION

Orchids are known as the metaphorical among of the most attractive global cut flower and pot plants in the world [1]. This highly aesthetic value plant also plays role as ecological indicator [2]. Orchids are belong to the Orchidaceae family which naturally grow mostly in tropical and subtropical forests, where they are largely epiphytic [3]. Specialists believed that the family Orchidaceae consists of the most species than other family of flowering plants exist with estimated 25, 000 species [4]. However, several species are currently at risk and listed as the endangered species [5]. This could be due to the low rate of propagation in nature and ongoing demand from human [6]. Hence, proper actions should be taken to sustain the existence of this plant.

The conventional method of orchids propagation is commonly through the division of axillary buds from the mother plant and very few are by seeds [7][8]. The seeds of orchids are tiny, lack of endosperm and stored food and have rudimentary embryos [9]. However, it is very inefficient and time consuming as it only produced one new growth in year [7][10]. *In vitro* technique has been invented as the alternative way for orchid to propagate large number of uniform clones which produced in short time and under proper condition compared to conventional method [11].

In vitro asymbiotic germination has become a favourite technique in a propagating orchids that are difficult to found in natural habitat and also rapid growth. The medium growth for germination orchid *in vitro* is complex, as all organic and inorganic nutrients and sugars are available without the fungus intermediary [12]. The complexion and sensitivity influence the better growth possibility for orchids. However, there are certain physical factors that influenced the biological behaviour during the development of plants in general such mechanical stresses [13]. This stresses could probably help to boost up the growth of seed germination.

Sound is a special form of mechanical stress and strong sound stimulation has an obvious effect on plant growth which has been proven to promote the growth of plant [14]. It was also reported that some sound wave could accelerate the growth of plants by changing the transition temperature of cell walls that were tightly related to the cell division of plant cells [15][16]. Besides, sound stimulation of low or high frequency are different according to the class of rhythm [17]. As reported by Singh A. (2013), plants are unable to distinguish between rhythmic and non rhythmic sound but are definitely showing positive effects on exposure to any kind of sound [18]. However, the material used in these former experiments is focused on effects of sound and specified on one species of plant, thus is not suitable for further understanding on relation of sound with plant.

In present study, different rhythms of music were used to test the effects on *in vitro* seed germination of 3 different species of orchids which are *Bulbophyllum longisepalum*, *Dendrobium tanii* and *Dendrobium anosmum*. This could provide further evidence on types of sound that give the best or worst growth rate towards plant growth.

MATERIALS AND METHOD

A. Seed Source and Sterilisation:

Mature seeds of *Bulbophyllum longisepalum, Dendrobium anosmum and Dendrobium tanii* were collected from Triang Botanical Valley Nursery, Pahang, Malaysia. Sterilisation on seeds was done initially by washing with running tap water for 30 minutes and soaked in 20% commercialized bleach containing 5.25% of sodium hypochlorite mixed with 2 drops of Tween 20 for 15 minutes [19]. The seeds were transferred into laminar air flow chamber under aseptic condition before vigorously swirled 3 times in distilled water. The seeds were dipped in 95% ethanol for 10 seconds, and then were flamed 5 seconds to ensure its sterility. Lastly, the capsules of the seeds were dissected longitudinally with a surgical blade to remove the seeds. The sterilized seeds were cultured on half strength Murashige and Skoog (MS) medium supplemented with 30g/L sucrose, 1 g/L activated charcoal, 2 g/L agar and 2 g/L peptone. The pH of the medium was set at range of 5.6 to 5.8. The media were autoclaved at 121oC for 15 minute under a pressure of 15 psi. The cultured seeds were placed under 25± 2oC in the dark.

B. Rhythm Stimulation:

The sound stimulation was generated by sound generating device [MP3 + medium size speaker]. The details setup of sound stimulation experiments were illustrated in Figure 1. 5 types of song from different rhythm were experimented; instrumental, ballad, yasin, rock and hiphop. The songs were used from the same size [320 kbps] under the same volume. The seeds were exposed to all these different rhythm with 5 replicates for each species. The treatments were done for 8 hours per day for 3 months. The control samples were placed in the same environment but without any rhythm treatment.

C. Experimental Design and Data Analysis:

Experiments were performed in 5 replicates for each species of orchids. One spatula of seeds was used for each

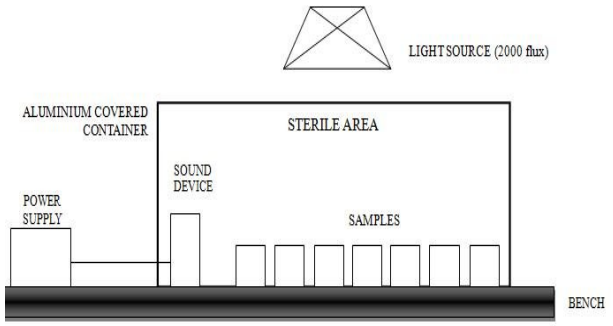

Fig. 1. The setup of sound stimulation

replicates and inoculated on to the medium prepared in 250 ml glass jar. The percentage of growth rate for seed germination and the length of plantlet were determined for each of the treatment. The germination and development of seeds were observed at 1 week interval within 12 weeks of study duration. The data were expressed as the mean (±) S.E. of three replicates. One-way analysis of variance (ANOVA) by using SPSS version 20 and Microsoft Excel 2007 were used.

RESULTS AND DISCUSSIONS

In the present study, the addition of activated charcoal was to improve the growth of orchid seeds germination. Activated charcoal has a very fine network of pores with large inner surface area on which many substances such as the toxic metabolites and brown exudation accumulation can be adsorbed, thus minimizing the possibilities for the explants from stunted growth and browning [20][21][5][22]. Peptone was also included in the media as it plays role in the seeds germination. According to Roy, (2001), in general peptone usage in orchid culture media is less common but it is applicable in a number of species by improving seeds germination and growth [23].

The development of seed germination and plantlet on *B. longisepalum*, *D. anosmum* and *D. tanii* were both studied during the 12 weeks of experiment. The maximum seeds growth rates for *B. longisepalum* (90%), *D. anosmum* (95%) and *D. tanii* (90%) were recorded from the exposure of Rock, Yasin and Ballad rhythm, respectively (Figure 2). The highest length of plantlets for both *B. longisepalum* (1.15cm) and *D. tanii* (0.38cm) were observed when exposed to Instrumental rhythm; meanwhile *D . anosmum* plantlets (0.55cm) was at maximum length when exposed to Yasin rhythm (Figure 3).
Figure 4 showed the seed development after 12 weeks in culture. In *B. longisepalum*, Rock rhythm showed the highest rate in growth and length of plantlets (Figure 4f). Yasin rhythm showed the second lowest of growth rate after Hiphop rhythm, with the shortest length of plantlets. The majoritycolor of protocorms in media treated with Yasin rhythm was whitish (Figure 4d). Meanwhile in *D. anosmum*, Yasin rhythm showed the highest seed germination growth at 95% (Figure 4j) and the lowest growth rate was observed in Control group at only 5% (Figure 4f). Even though Rock rhythm of *D. anosmum* showed low growth rate, the highest plantlets length was measured from this group (Figure 4l). Figure 4o showed *D. tanii* with the highest growth rate when exposed to Ballad rhythm. However, the highest length of *D. tanii* plantlets was observed from medium treated with Instrumental rhythm (Figure 4n). No germination of *D. tanii* seeds was observed in both media treated with Hiphop and Rock rhythm (Figure 4q and 4r).

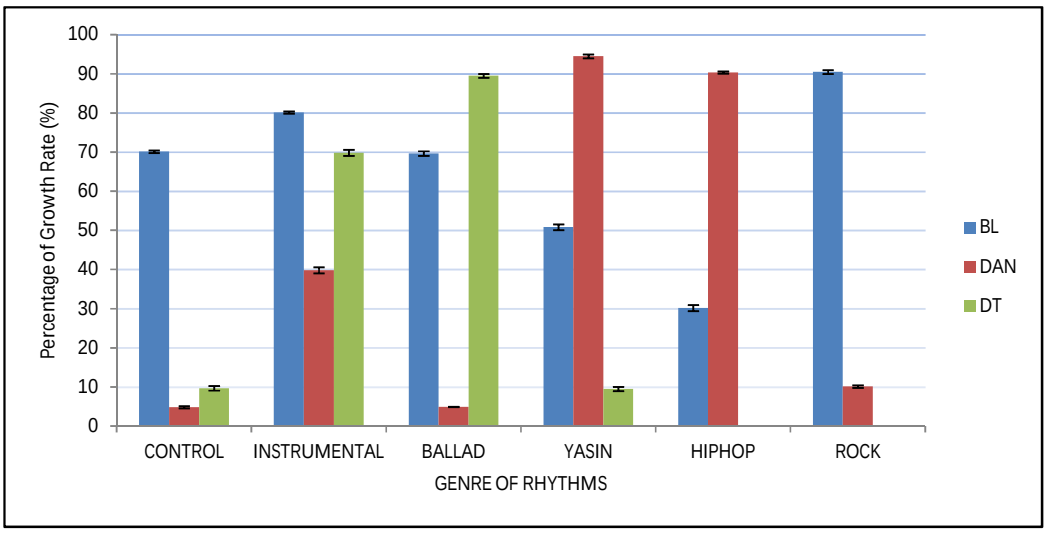

Fig. 2. Effect of various rhythms on the growth rate of in vitro seed germination of *B. longisepalum* (BL), *D. anosmum* (DAN), and *D. tanii* (DT). X-axis represents the treatment using music from genres of Instrumental, Ballad, Yasin, Hiphop and Rock. Control group served as the standard. The seeds were cultured using half MS strength supplemented with 30g/L sucrose, 1 g/L activated charcoal, 2 g/L agar and 2 g/L peptone and cultured under the dark for 8weeks and under the light within 4 weeks. Percentage of growth rate was scored after 12 weeks of culture. Also represents a total 5 replication per treatment.

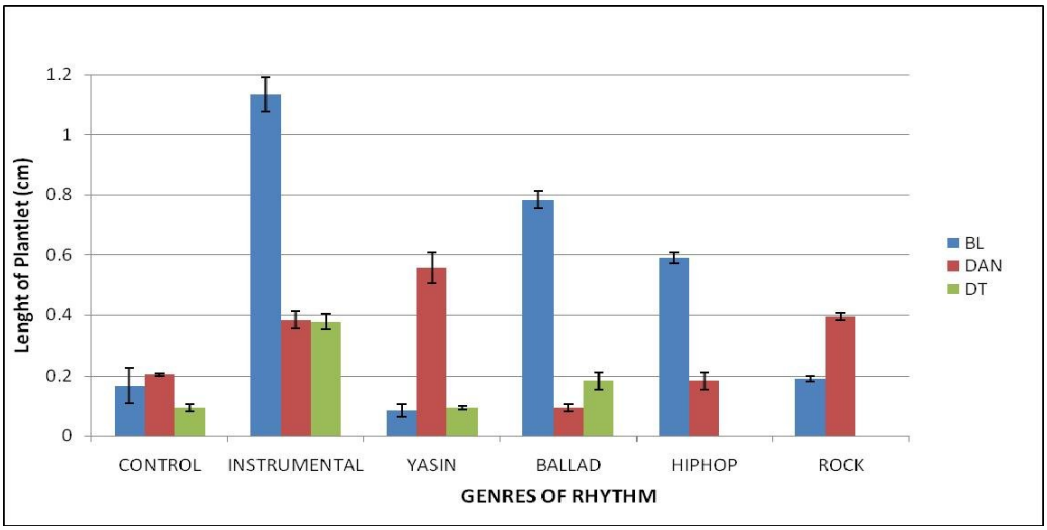

Fig. 3. Effect of various rhythms on the length of plantlet (cm) formed from *B. longisepalum* (BL), *D. anosmum* (DAN), and *D. tanii* (DT). The length was measured by using measurement device. x-axis represents the treatment with music from genres of Instrumental, Ballad, Yasin, Hiphop and Rock. Control group served as the standard. The seeds were cultured using half MS strength supplemented with 30g/L sucrose, 1 g/L activated charcoal, 2 g/L agar and 2 g/L peptone and cultured under the dark for 8 weeks and under the light within 4 weeks. Percentage of growth rate was scored after 12 weeks of culture. Also represents a total 5 replicated per treatment.

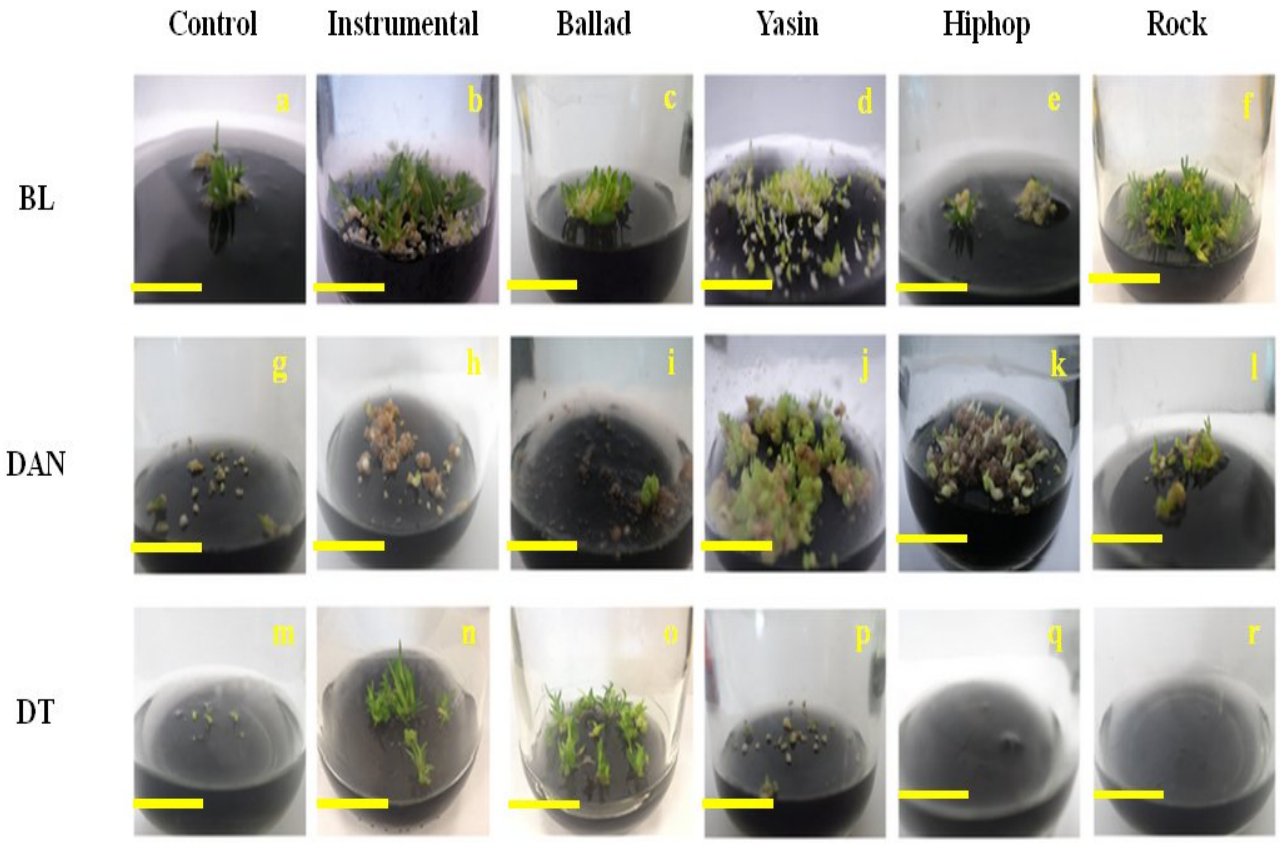

Fig. 4. Observations of *B. longisepalum* (BL), *D. anosmum* (DAN) and *D. tanii* (DT) seeds development after 12 weeks of culture. The sound treatments were represented by Instrumental, Ballad, Yasin, Hiphop and Rock rhythm. Control groups were served as the standard. **a:** Seeds were germinated to form greenish protocorms at Week 2. Plantlets showed 1-2 leaves were formed at the Week 12 of culture (bar = 0.5cm). **b:** Seeds were germinated into greenish protocorms at Week 2 of culture. Plantlets with leaves were seen after Week 10 and continue to intrude more at Week 12 of culture (bar = 1.0 cm). **c:** At Week 2 of culture, only few of seeds were germinated to greenish protocorms. Plantlets were formed at Week 10 and leaves were seen at Week 12 of culture (bar = 1.0cm). **d:** Protocorm formation was observed at Week 2. At Week 8, majority of protocorms turned to whitish and showed very minimal height of plantlet (bar = 0.5cm). **e:** Formation of very few greenish protocorms noted at Week 2 and plantlets were formed at 10 Week of culture (bar =1.0cm). **f:** Yellowish and greenish protocorms were formed at Week 2 .Plantlets with first leaf were formed at Week 10 and continue to project leaves until Week 12 of culture (bar = 1.0cm). **g:** Only several spots of seed were germinated to form brownish protocorms which were observed at Week 2. Plantlets were formed at Week 12 of culture (bar = 0.5cm). **h:** Brownish protocorms were formed at Week 3 and very few plantlets were formed after 10 week of culture (bar = 0.5cm). **I:** One spot of seeds inoculated were germinated to greenish protocorms at Week 3 of culture and plantlets were formed at Week 12 of culture (bar = 0.5cm). **j:** Seeds of yellowish dust-like were germinated to uniform greenish protocorms at Week 2 and plantlets were observed at Week 10 of culture (bar = 1cm). **k:** Protocorms were formed at Week 2 of culture. Plantlets were formed at Week 10 and leaves were noted at Week 12 of culture (bar = 1.0cm). **l:** Greenish protocorms were seen at Week 2 of culture. Plantlets were formed at Week 10 and leaves were seen at Week 12 of culture (bar = 1.0cm). **m:** A few plantlets were formed at Week 12 of culture. Greenish and whitish protocorms were noted at Week 2 of culture (bar = 0.5cm). **n:** Formation of greenish protocorms were noted at Week 2 and growth of plantlets were observed at Week 8 of culture. The protrusion of 3 - 4 leaves were seen at Week 12 of the culture (bar = 1.0cm). **o:** Seeds germination started with swelling- like greenish protocorms formation at Week 2. Protocorms were observed at Week 4 of culture. At Week 10 of culture, plantlets were formed and 3-4 leaves/plantlet were noted at Week 12 (bar = 1.0cm). **p:** Seeds were germinated to greenish protocorms at Week 2 and changed to brownish at Week 8. Very few plantlets were formed at Week 12 (bar = 0.5cm). **q & r:** No germination progress was observed during the 12 weeks of culture (bar = 1.0 cm). All of the experimented seeds were cultured on half strength Murashige and Skoog (MS) medium supplemented with 30g/L sucrose, 1 g/L activated charcoal, 2 g/L agar and 2 g/L peptone. The samples were placed under the dark for 8 weeks and 4 weeks under the light of 2000 flux

As we studied on *D. anosmum* of Yasin rhythm, the second week after the inoculation, the majority of seeds were seen swollen from yellowish dust-like color protocorm to greenish protocorm and continue to grow until Week 12 of the experiment with the formation of leaves on majority of the plantlets. While in the Control group of D. anosmum which was not treated to any rhythm, only 5% of the seeds were seen germinated and formed greenish color protocorms after the third week of culture and remained in protocorm form without any formation of new shoots or leaves until Week 12.

From the results analyzed, the rhythms exposure towards seeds germination has the tendency to give positive effect in most of the groups. This has been supported by a study done on plants responded to sound which increase the speed of growth and gave better size of plant [24]. As we can see, the highest rate of seed germination of different orchids was seen in different rhythms. This statement also has been reported by previous studies whereby, music makes seed germination grow faster but the response is quite specific [25][18]. According to Singh A. (2013), plants were unable to distinguish types of sounds. Thus, this indicates that sound gave different effects on seed germination on different species of orchids [18].

The incorporation of Ballad rhythm on *D. tanii* gave significant effect during seeds germination. Nevertheless, it seems Rock and Hiphop rhythms inhibited the growing process of same seeds species. There was no significant change on *D. tanii* seeds in response to both Rock and Hiphop rhythm started from the Week 1 until Week 12 of culture (Figure 4). This could be due to incompatibility on type of rhytm for this particular orchid species and interruption during the growing process. Several studies reported that, slight stress could cause damage or promote the division on plant cells [26][27][8]. The different musical elements used gave different sound wave frequencies and produce various stimulations towards the plants [25]. This explained at certain frequencies, sound can promote changes in cells, thus enhance the cell division and plant growth. It can also reduce or completely inhibit the plant growth. This theory also has been supported by several studies that explained on how sound stimulation of certain frequency promotes the fluidity of membrane wall, hence gives better absorption of nutriment to benefit the growth cell during seed germination [28][29].

Some of the protocorms germinated showed brownish color mostly in Control group, Hiphop, Ballad and Instrumental rhythms of *D. anosmum*. This occurrence could be attributed to the toxic metabolite exudates from the growing protocorms. As reported from American Orchid Society [30], Dendrobium commonly known as the diverse genus orchids with different growth phases of each species, hence this might be the reason of the difference observations were noted between *D. anosmum* and *D. tanii* seed germinations. Meanwhile, a study done by Islam (2011), the requirement of nutrients as well as metabolic activities varies depending on growth stages [31].

CONCLUSIONS

In vitro micropropagation of several species orchids can be done by culturing sterile seeds into suitable medium. Different types of rhythm when exposed to the seeds give different effect towards the rate of germination. Based on the findings from this study, it is assured that plants are definitely influenced by the sound in term of rhythms. However, different plant species required different rhythm for their best growth. The success in finding best influence of rhythm on plant growth will enhance the orchids cultivation business. The study should be continued on testing different species of plants with different types of music.

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