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Effect of Different Sound Genres on *In Vitro* Seed Germination of *Grammatophyllum Hybrid* and *Grammatophyllum Stapeliiflorum* Orchids

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Abstract: Auditory stress has been known to affect the development of plants. Recent findings have shown that the stress produced by music induced a positive effect on plants growth. However, different types of music have different kinds of stimulation on plant development. The aim of this study was to examine the *in vitro* seed germination of two *Grammatophyllum* species when exposed to five different music genres. The *Grammatophyllum* species used were *G. hybrid* and *G. stapeliiflorum* while the music genres were Instrumental, Rock, Hip hop, Yassin (Quranic reciting) and Ballad. Green capsules of *Grammatophyllum hybrid* and *Grammatophyllum stapeliiflorum* were obtained from Triang Botanical Valley, Pahang, Malaysia. Each group of seed cultures were exposed to different music genre for 8 hours starting from 9 am until 5 pm, each for a 6 months period. Seed cultures were kept in the dark for the first 3 months. One untreated (no music) group was kept as a control. All cultures were maintained on half-strength Murashige and Skoog (MS) media supplemented with 30 g/L sucrose, 0.5 mg/L 6-benzylaminopurine (BAP), 2 g/L peptone, 1 g/L activated charcoal and 2.5 g/L gelrite. Observations were recorded every month for the number of shoots emerged and shoot length. Data was analysed by using a one-way ANOVA. At the end of the experiment, it was found that music exposure had a positive effect on the seeds' germination as compared to the untreated control group. For *G. hybrid* species, the highest shoots number counted was 19.33±3.79 which was observed on seeds exposed to Yassin. The highest shoot length measured was 2.02±1.10 cm when it was exposed to Rocks music. In contrast, *G. stapeliiflorum* species showed the highest number of shoots of 12.00±2.64 when exposed to Ballad and the highest shoot length achieved by this species was 0.99±0.38 cm when it was exposed to Instrumental music. Analysis by using Kruskal-Wallis H and one-way ANOVA showed that $p < 0.05$ for all parameters. The findings showed that different species of orchids need a different type of music to influence the rate of its germination and growth.

Keywords: *Grammatophyllum hybrid*, *grammatophyllum stapeliiflorum*, *in vitro*, music exposure, orchids, seed germination.

INTRODUCTION

Plant Acoustic Frequency Technology (PAFT) an acoustic biology study which used sound technique through sound wave generator produces sound that complements the frequency of a certain resonance of a plant to stimulates plant growth, boost crop production, upgrade the quality of plants and improves disease endurance [1, 2]. Studies conducted by Qingwu *et al*. [2] have shown that flowering, fruiting and chlorophyll content of pot-bred strawberry can be increased by using the PAFT technique. The study compared the effect of net photosynthetic rate, chlorophyll content and chlorophyll fluorescence parameters when strawberries were treated with acoustic waves. Their finding concluded that acoustic waves affected the growth of strawberry plants in direct proportion to the treatment time. This finding was similar to finding found in rice, cucumber and lettuce, as reported by Hou [3].

The beneficial usage of music in the agricultural field has also been proven in various studies. One study, conducted

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by Creath & Schwartz [4], looked at the relationship between music and seed germination of black zucchini squash (*Cucurbita pepo*) and Clemenson spineless okra (*Hibiscus esculentus* / *Abelmoschus escelentus*) seeds. Her study measured biological effects of music, noise, and healing energy on non-human subject to prevent human preferences and placebo effects by using seed germination as an objective biomarker. Five series of experiments were conducted where germination of zucchini and okra seeds was monitored under the influence of manipulative agents such as the musical sounds, noise and healing energy. The seeds were alternately swapped around in between two/four trials to counter-balance the agent conditions. Her findings concluded that seeds sprout faster when they are exposed to musical sound as compared to the noise treatment and untreated. She further discussed that, although both musical sound and noise had been adjusted to have equal amount of sound and vibration, yet, musical sound still show a positive effect. She reasoned that musical sound was dynamically arranged and the acoustic wave changes with time while noise wave was constant. The musical sound also comprises of melody organization, pauses and rhythm that are said to mimic the healing energy. However her study did not include any of the healing energy itself.

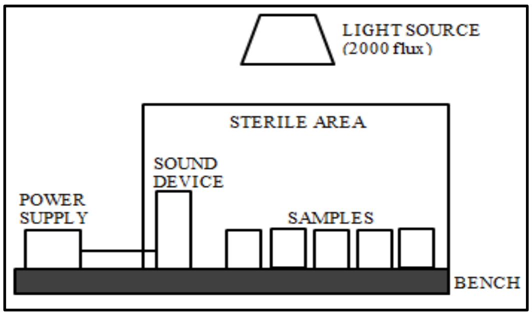

Fig. (1). The experimental setup for every group. Samples were enclosed inside a sterile germination chamber and exposed to a different music genre.

Findings from Creath & Shwartz [4] were also parallel to observations from Singh *et al.* [5] on the growth of common bean plant (*Phaseolus vulgaris*) where it showed a positive outcome when the plant was exposed to music. However, Sing *et al.* [5] claimed that a plant cannot distinguish between “music” and “noise” as both violin and noise sound result were better outcome compared to the control set. On the other hand, Vanol & Vaidya [6] study stated that the plants were able to differentiate between sound and noise, given that the alternate frequencies exist in the sound given to plants. This was done to manipulate the mechanical perturbation surrounding the plant’s environment. Thus, traffic noise was stressful for plants and led to the slower rate of plant’s growth compared to classic and rock music. His study on common guar or cluster bean (*Cyamopsis tetragonoloba*) showed that seed germination and plant growth were better when exposed to high frequency (1500-2000) sound.

The same result was observed in seeds exposed to traffic noise. At this point, it seems that all kind of auditory exposure brings out the plant growth potential. However, Chivukula & Ramaswamy [7] argued that certain types of music can have damaging effects on plants. Their study on *Rosa chinensis* revealed that plant growth in the control set was higher than the plant growth subjected to rock music. The study showed that the shoot and internode length of *Rosa chinensis* was at the lowest when exposed to rock music. Observation also showed that the plant bent away from the source of the rock music and the number of the leaves also decreased throughout the experimental period. The plant displayed dull appearance after 3 weeks of exposure and the frequency of the flowering and diameter of the flowers were also at the lowest among other music types. Another highlight from their finding stated that plants that were exposed to rock music were the first to develop thorns. Based on these findings they concluded that the vibration coming from the rock music had negative effects on plant growth.

Grammatophyllum is a genus of 12 known species. Its populations are spread in Malaysia, Sumatra, Indonesia, Java, Celebes, Philipines, Singapore, Myanmar, Papua New Guinea and Thailand and they usually live at elevations of 200 to 1000 meters. Three of the *Grammatophyllum* species are found in Malaysia. These species are *G. stapeliiflorum*, *G. speciosum* and *G. kinabaluense* which were also commonly known as the tiger orchid for its flower patterns resembled the pattern of a tiger’s stripes and are loved by orchid lovers. *G. stapeliiflorum* is exceptionally rare in cultivation due to its elite requirement, hence making it more desirable to private collectors. Moreover, since 1975, *Grammatophyllum* has been listed as an endangered species by the Convention on International Trade in Endangered Species (CITES) which signify the probability for this genus to become extinct if trading is not controlled. Similar to other orchids, *Grammatophyllum* are accustomed to a lengthy immature period before developed into flowers. Their maturation time ranged from two to several years which makes it a valuable orchid among orchid lovers and breeders [8 - 10]. Moreover, the pods of orchids produced numerous minute seeds that have no endosperm and lack of nutrient for germination. This makes orchid seed germination far more challenging when germinating in their natural habitats. Although orchids are able to produce up to 4 million seeds per capsule, there is no guarantee that every single one of the seeds will germinate and flower during the breeding season. The seeds also require appropriate symbiotic fungi to germinate as they do not have the capacity to produce their own growth supplement.

Thus, *in vitro* seed germination might be the answer to produce *Grammatophyllum* plantlets in mass quantity. This technique is an alternative way for mass propagation of uniform clones where plant products can be yielded in a short time and under similar conditions as of natural seed germination. *In vitro* asymbiotic germination has become a new trend in propagating orchids as orchids are difficult to find in their natural habitat. The technique also makes possible for faster growth rate of orchids due to its controlled environment. The media used for orchid germination in *in vitro* are unique, as all organic, inorganic nutrients and sugars are provided directly without the fungus intermediary [11]. By practising this technique, the seeds are supplemented with enough nutrients and carbon sources to induce germination asymbiotically [12 - 14].

Many researches have been conducted in order to develop the best protocol for orchid germination by means of tissue culture technique. For example, Chen *et al.* [15] developed asymbiotic seed germination protocol for *Paphiopedilum spicerianum*. They used modified MS and Robert Ernst media in which they reduced the amount of agar and sucrose. In their study, they tested the effect of reduced salt concentration and the addition of coconut water as a supplement for the orchid seed germination. From their experiment, they found out that ¼ strength modified MS supplemented with coconut water and modified Robert Ernest media and addition of activated charcoal and coconut water were the best media combination for obtaining viable protocorms of *Paphiopedilum spicerianum*. The team also noted that there was a connection between pretreatment duration, medium and light sources in the process of seeds germination. Apart from asymbiotic *in vitro* seed germination, there was also study done by Tan *et al.* [16] that showed protocol of *in vitro* orchid seeds germination by means of fungi infections. Two strains of *Tulasnella sp* were isolated from the roots of *Dendrobium nobile* which were later grown and used in germination of *Dendrobium officinale* seeds. The media used in this study was oat meal agar which was inoculated with strains of *Tulasnella sp*. Tan *et al* findings revealed that strains of *Tulasnella sp* gave positive effects for seeds germination with 98.47% and 99.05% success as compared to the control (81.05%). They also mentioned that the fungi infection promoted seed development up to stage 5, while asymbiotic germination can only develop up to stage 2. Both reports showed that there were plenty of ways to propagate orchid seeds successfully either through symbiotic or asymbiotic germination. By practising tissue culture techniques, the germination percentage of orchid seeds can be increased as compared to the conventional method. Not only that the technique managed to shorten the time to culture orchid plants, it also allows utilizing every single piece of seed obtained from the capsule, thus maintaining ample supply of orchids for commercialization.

Although germination of orchid seeds is guaranteed when given suitable compositions of growth media, it still depends on several factors that affect the plant growth rate. One of the factors is mechanical stress, or vibration produced by music [12, 17]. In this study, five different music genres were tested to study their effect on *in vitro* seeds germination of *Grammatophyllum hybrid* and *Grammatophyllum stapeliiflorum* plant.

MATERIALS AND METHODS

Capsule Seed Sterilization

Green capsules of *Grammatophyllum hybrid* and *Grammatophyllum stapeliiflorum* were obtained from Triang Botanical Valley, Pahang. Capsules were thoroughly rinsed under running tap water for 30 minutes to remove soil and other contaminant agents. The capsules were then transferred into 1% sodium hypochlorite solution with the addition of 3 drops of Tween 20. The capsules were left inside the mixture for 15 minutes with gentle agitation and then rinsed with sterile distilled water for three times to completely remove the leftover sterilant. After that, the capsules were immersed in a 95% denatured alcohol for 30 seconds, and the outer coats were flamed. Sterilized capsules were cut in half and by using a sterile spatula, a little bit of the seeds were stockpiled at the tip of the spatula and inoculated on the surface of solidified media. Seeds were randomly spread onto the surface of media.

Media Culture

The seeds were inoculated on the surface of media inside a 250mL glass jars. Media used in this experiment was half-strength Murashige and Skoog (MS) media supplemented with 30 g/L sucrose as a carbon source. To enhance the germination of seeds, 0.5 mg/L 6-benzylaminopurine, 2 g/L peptone and 1 g/L activated charcoal were added [18]. 2.5 g/L gelrite was added to the solidify media. The pH of the media was altered to 5.4-5.8 and autoclaved at 121°C for 15 minutes.

Fig. (2). Pictures of protocorms developed after the first 3 months of the experiment. (**A**) green protocorms of *Grammatophyllum hybrid*. (**B**) white translucent protocorms of *Grammatophyllum stapeliiflorum*. (**C**) brown protocorms of *Grammatophyllum stapeliiflorum*. Note that *G. hybrid* seeds developed into large spherical protocorms while *G. stapeliiflorum* seeds developed into smaller spherical protocorms.

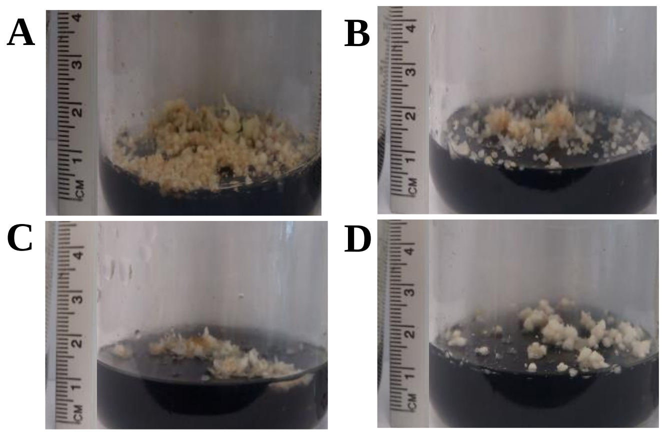

Fig. (3). Pictures taken by the end of the experiment of protocorms that did not produce any shoot. (**A**) White and brown protocorms of *G. hybrid* that were exposed to instrumental music for 6 months and did not show any sign of shoot formation. (**B**, **C**) Brown protocorms of *G. stapeliiflorum* that were exposed to Hip Hop and Yassin music for 6 months, with no shoots being observed. (**D**) White protocorms of *G. stapeliiflorum* that were untreated (control group), with no shoots being observed.

Music Exposure

The genres/song used in this experiment were Ballad (Because I Miss You - by Jung Yong Hwa), Hip hop (The Real Slim Shady-by Eminem), Instrumental (Caravansary-by Kitaro), Rock (Numb Encore-by Linkin Park), and Quranic recitation (Yassin verse). One group with no music treatment was used as a control, which was not exposed to any music. The music exposure was given 8 hours a day from 9 am until 5 pm for 3 months. The exposure of music was generated by medium-sized MP3 speakers and the volume was set at level 7 for each group.

Experimental Design and Data Analysis

The jars containing the seeds were divided into 6 groups, with each group having 3 jars from each species. Each group represented one genre of music. One group was reserved as a control and was not exposed to any music. Experiments were done in triplicate for each species. The jars on each group were placed inside a germination chamber with identical MP3 players playing only one selected genre throughout the experiments. Fig. (**1**) shows the setup for the experiment. Observations were carried out monthly after the seeds germinated. The parameters used to analyse the experiments were the number of shoots germinated and the length of shoots. The colour of protocorms were also observed and recorded throughout the study. The data were reported as the mean±SD for each parameter mentioned above. Analysis was done by using IBM SPSS Statistic 20 software with *P* values of 0.05 or less was considered as significant. Graphs were plotted to show the growth rate of each orchid under different types of music genre.

RESULTS

The experiment was performed and observed in a six-month period. There was no growth for the first three months, but green, white and brown protocorms were observed (Fig. **2**). Green protocorms formation were mostly observed in *G. hybrid* species while yellow and translucent white protocorms were observed in *G. stapeliiflorum*. Towards the fifth month of the experiment, white protocorms from *G. stapeliiflorum* had developed into light greenish colour. After three months of the experimental duration, shoots were first observed emerging from the green protocorms that were exposed to Yassin. This was followed by seeds that were exposed to Rock and Hip hop music. The following month, shoots started to emerge from the Instrumental music group and also from the Control group. The last shoots to emerge were from the Ballad music group. Most shoots were developed from the green coloured protocorms with the exception for *G. hybrid* where the shoots that were exposed to Ballad music developed from the white protocorms. By the end of the experiment, there were more white coloured protocorms observed as compared to the yellow protocorms. All previous green protocorms had successfully elongated and formed shoots. However, no significant changes were observed on the brown coloured protocorms. Fig. (**3**) showed the white protocorms of *G. hybrid* and mixtures of white and brown protocorms of *G. stapeliiflorum* species that failed to produce any shoot by the end of the experiment.

Fig. (**4**) shows plants from all experimental groups. Both plant species illustrated morphological changes from seeds to protocorms and lastly to shoots. Some samples developed into lateral shoots and roots at the end of the experiment. All seed cultures were maintained on half strength Murashige and Skoog (MS) media with the addition of 30 g/L sucrose, 1 g/L activated charcoal, 2 g/L peptone and 2.5 g/L gelrite.

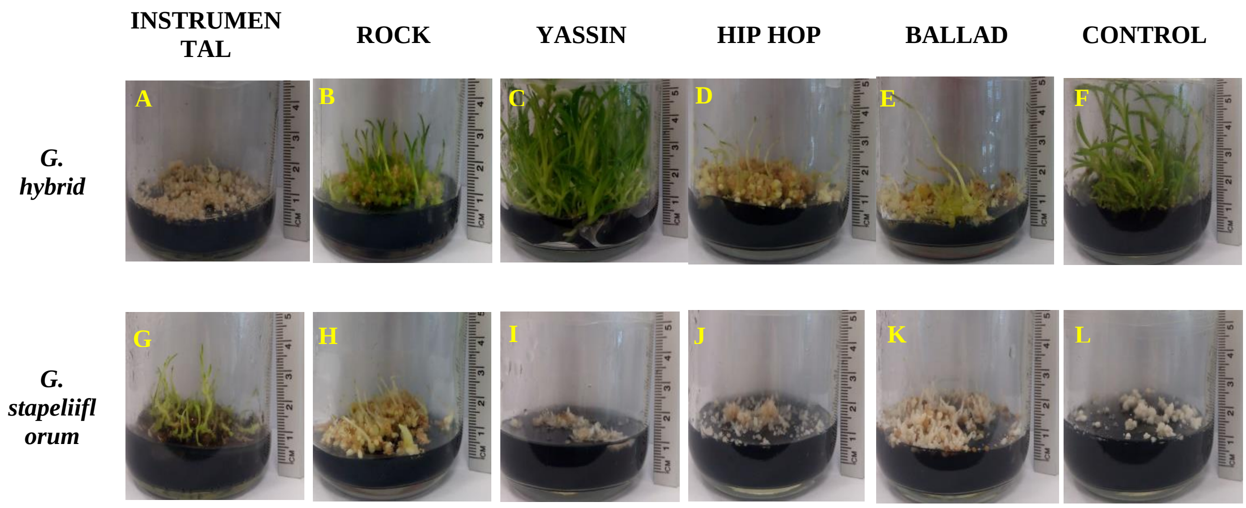

Fig. (4). Pictures of *G. hybrid* and *G. stapeliiflorum* seed germination after 6 months of culture. The exposure of different music genres was categorized as Instrumental, Rock, Yassin, Hip hop, and Ballad. Control groups served as a standard.

The following are the description of each group observation based on Fig. (**4**) :- (**A**) Seeds were cultured and started to formed white protocorms in the 3rd month. Until the end of the 6th month, no shoots were observed. (**B**) On the 3rd month, green shoots started to emerge from the green protocorms of *G. hybrid*. Emerged shoots were sturdy with 4 shoots formed. By the end of the 6th month of the experiment, the tallest sturdy shoot had measured ~3.00 cm in length. (**C**) ~15 thin shoots were formed on the 3rd month of the culture. Leaves started to be observe on the 4th month of the culture. On the 6th month, lateral shoots had formed and white roots were observed from several of the shoots. (**D**) Many thin shoots formed on the 3rd month of the culture. Leaves were starting to be observed on the 5th month. Lateral shoots and white roots were observed during the 6th month of the culture. (**E**) Seven sturdy shoots emerged on the 4th month of the culture. On the 6th month, formed shoots were almost ~2.0 cm in length. (**F**) Green protocorm formation was observed from 2nd and 3rd month. On the 4th month, sturdy green shoots had emerged with a length of about ~1.0 cm. On the 6th month, three white roots were visible. (**G**) Seeds started to show protruding of the shoot on the 4th month. Many shoots emerged with a green leaflet on the 5th month and the shoots formed were thinly built. By the 6th month, lateral shoots and white roots could be observed on some of the plantlet. (**H**) White and brown protocorms were observed on the 3rd month of culture. On the 5th month, more than 15 shoots were emerged. Leaves of shoots were starting to show on the 6th month. (**I**) Shoots were not formed until the 6th month of the culture. Formed seeds were white and brown protocorms accumulated together and formed a clump. After the 6th month, tips elongating from white protocorms were observed but no shoots had developed. (**J**) Seed development was slow. Brown coloured protocorms only started to be observed on the 3rd month of experiment. By the end of the experiment, mixtures of white and brown protocorms were recorded. However, no shoots were formed. (**K**) White and brown coloured protocorms were observed throughout the 2nd and the 4th month of the culture. Many thin shoots coming out from the white protocorms were observed on the 5th month of the culture. (**L**) Seeds formed white protocorms from the 2nd until the 6th month of the culture. However, no shoots were formed.

Fig. (**5**) showed graphs for the number of shoots of *Grammatophyllum hybrid* and *Grammatophyllum stapeliiflorum* at the end of the experiment. The highest number of shoots calculated for *G. hybri*d species was 19.33±3.79 which was observed from the Yassin group. The second highest shoot number was obtained from the Hip hop group. However, there were big differences in the value between the Yassin and the Hip hop group (7.33±4.93) shoots. Meanwhile, the shoots counted for Rock and Ballad groups were 5.33±2.51 and 2.67±0.58 respectively and there was no shoot germinated from the instrumental group as only brown protocorms were observed. For the *G. hybrid* control group, 3.33±0.58 shoots were counted (Fig. **5A**). A descriptive test performed by using SPSS Statistic 20 software showed abnormal data distribution and further analysis was done by using a Kruskal-Wallis H test. Analysis showed significant differences in the number of shoots of *G. hybrid* (Chi-square : 15.071, df : 5, P-value<0.05).

On the other hand, sprouted seeds were observed in *G. stapeliiflorum* that were exposed to Ballad, Instrumental and Rock music while seeds exposed to Hip hop and Yassin did not show any shoot formation apart from brown protocorms formation. There was no shoot development from the control group either. The highest number of shoots was 12.00±2.65 obtained from the Ballad group. Instrumental and Rock groups recorded 7.67±1.53 and 7.33±2.52 number of shoots respectively (Fig. **5B**). A descriptive test performed by using SPSS Statistic 20 software showed abnormal data distribution and further analysis was done by using Kruskal-Wallis H test. Analysis showed a significant difference in the number of shoots of *G. stapeliiflorum* (Chi-square : 16.064, df : 5, P-value<0.05).

Fig. (**6**) shows a graph of the length of shoots of *G.hybrid* and *G.stapeliiflorum.* For *G. hybrid*, the highest shoot length was measured from the Rock group where the length was 2.02±1.10 cm. Meanwhile, other groups have almost similar measurements with each other with differences between each group being 0.2 cm. For the Ballad group, the shoot lengths were 1.69±0.61 cm, followed by the Hip hop group with 1.46±0.91 cm and the Yassin group with 1.28±0.75 cm. However, there was no significant growth in the Instrumental group. No shoots were formed from any sample that was exposed to the Instrumental music though green protocorms were spotted. Meanwhile, the untreated control group for *G. hybrid* yielded shoot lengths of 1.13±0.80 cm (Fig. **6A**). A descriptive test performed by using SPSS Statistic 20 software showed normal data distribution and further analysis was done by using a one-way ANOVA test. One-way ANOVA analysis showed that music exposure significantly affected the length of shoots measurement in *G. hybrid* species (F = 5.815; df = 5, 109; P<0.05). For *G. stapeliiflorum*, there was only a 0.04 cm difference between the highest and second highest shoot length measurement. The Instrumental group achieved 0.99±0.38 cm shoot length while the Rock group achieved 0.95±0.43 cm. For the Ballad group, the length of shoots measured 0.58±0.17 cm. No shoots developed in other groups and only brown protocorms were observed throughout the experiment (Fig. **6B**). A descriptive test performed by using SPSS Statistic 20 software showed abnormal data distribution. Kruskal-Wallis H analysis showed that music exposure significantly affected the length of shoots measurement in G. stapeliiflorum (Chi-square : 16.265, df : 5, P-value<0.05).

DISCUSSIONS

From this experiment, we observed that orchid seeds developed into protocorms first before forming into shoots. This phenomenon is normal as orchid's seeds have a small embryo and lack of endosperm. In their natural habitat, orchids depend upon fungal infection for a supply of carbohydrates, nutrients and water for the seeds' development [19, 20]. The supply of nutrients from the fungal colonization promotes seed germination, protocorm development and seedling growth in orchids [21]. However, in this study, as we implemented *in vitro* cultures for orchid seeds germination, there was no need for the fungi inspection as Murashige and Skoog media supplied all the necessary nutrient. Thus, seeds of orchids were able to progress into protocorms in a three month period as compared to a year naturally [22].

Protocorms are well-differentiated tissues that are able to grow into two apparent structures, which are shoot and root meristems. These structures could easily develop into plantlets when transferred into a plant growth medium [23]. Most shoot developed in our study was formed from green coloured protocorms. This showed that green protocorms might be the precursor for the emergence of orchid's shoots. However, by the end of the experiment green protocorms of *G. hybrid* exposed to Instrumental music did not developed into shoots. It was noted that it might take longer than six months for the shoot to develop. Therefore, a longer period of experiment is advised for future study. The shifting in the colour of the seeds such as white, yellow and brown might be due to the proliferation and testa breaking of the seeds where the seeds have to absorb water and nutrient to generate cells, which then lead to the formation of globular shaped arrangement known as protocorm [13].

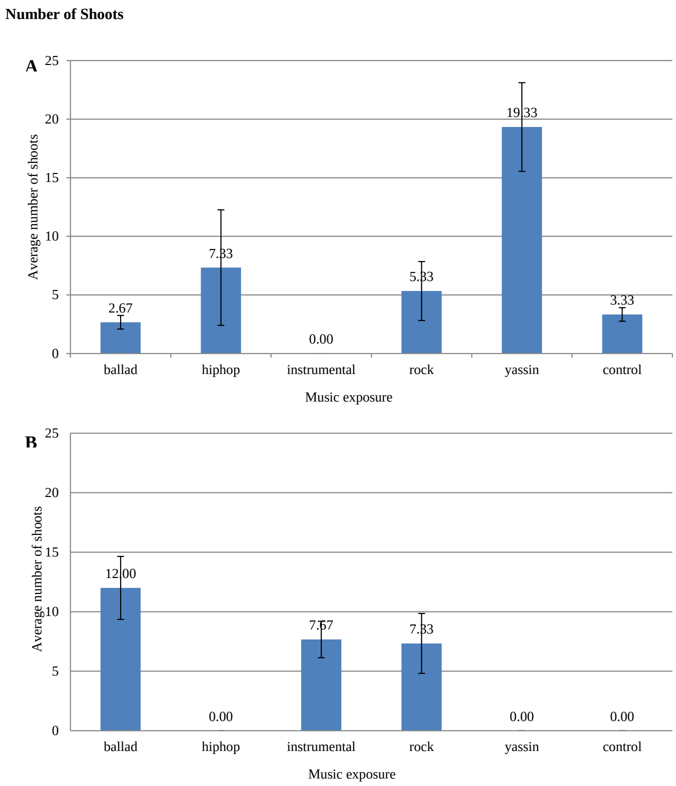

Fig. (5). Effects of different music exposures on the number of shoots developed for *G. hybrid* (**A**) and *G. stapeliiflorum* (**B**). Graphs were plotted based on the calculated shoot numbers in the final month of the study. All results were expressed as mean ± SD.

In this study we also noticed the inability of white and brown coloured protocorms to develop into shoots. One possible reason for this is the experimental period might be insufficient for white protocorms of *G. hybrid* exposed to instrumental sound to produce shoots. It might take a while before new shoots start to develop as green and white protocorms are viable. On the other hand, brown protocorms might be an indication that shoot formation is impossible as there was no shoot formed from any brown coloured protocorms. This showed that *G. stapeliiflorum* did not favor the stress/vibration coming from Hip hop and Yassin sound, thus resulting in more brown coloured protocorms inside a jar. Since there were no green cells formed in neither groups, we can assume that Hip hop and Yassin sounds were affecting the chlorophyll formation of the seeds during the cell expansion into protocorms.

It is not clear to us why the seeds failed to germinate but it can be due to the pretreatment that was used on the capsules of the seeds. Zeng *et al.* [24] on his study claimed that the failure of asymbiotic seed germination was caused by the physiological or mechanical mechanisms that maintain seed dormancy. The dormancy of a seed can be broken by temperature or by the usage of chemicals such as Ca or NaOCl during pretreatment. The maturity of orchid seeds can also affected the rate of seed germination of certain orchid species. It was recorded that immature seeds provided a better germination rate than mature seeds since the integuments become more impermeable to water as the seeds matured [25].

Based on the previous and present studies, it was concluded that sound waves could affect the plant's growth rate physiologically and biochemically [26]. The plants immobility exposed them to the risks of the environmental stimuli such as rain, wind and magnetic field. To protect themselves, plants need to be able to adapt to their surrounding by actively altering their outer appearance, inner structure and physiological nature to give them better survival [27]. When plants were exposed to audio frequency, gene expression for peroxidase isoenzyme was activated, and caused the process of gene transcription and translation to change [28]. The exposure also increased the content of the plant's growth hormones such as indoleacetic acid (IAA) and polyamine compounds [29], thus stimulating the cell division and construction of vascular bundles, leaves and flower buds [30]. Other than that, the study by Qingwu *et al.* [2] also confirmed that sound waves initiated a sensitive response of the photosynthetic process, increased endocrine levels in relevance with energy and metabolism, and changed the conformation of cell membranes. This was supported by Wang *et al* [31] research regarding the membrane fluidity, activity of ATPase of the plasmalemma, and growth of callus tissue. The explanations for enhanced photosynthetic capacity of music-exposed plants lie in two aspects. The first one was factors comprising of absorption, transformation and transfer of light energy, such as degradation of chloroplast pigment, intensification of electron transfer and photochemical efficiency resulted by increased light-harvesting pigment complex. The second aspect is about factors concerning carbon assimilation, including stomatal conductance, rubisco activity and attenuated mesophyll resistance which results from raised applicable enzyme activity in the Calvin cycle [2]. Although mechanical stress induced by sound waves gave no obvious changes on the plant's DNA, it influenced the acceleration of RNA synthesis and soluble protein, which resulted in the increased level of transcription, hence more proteins are produced for plant growth and survival [27].

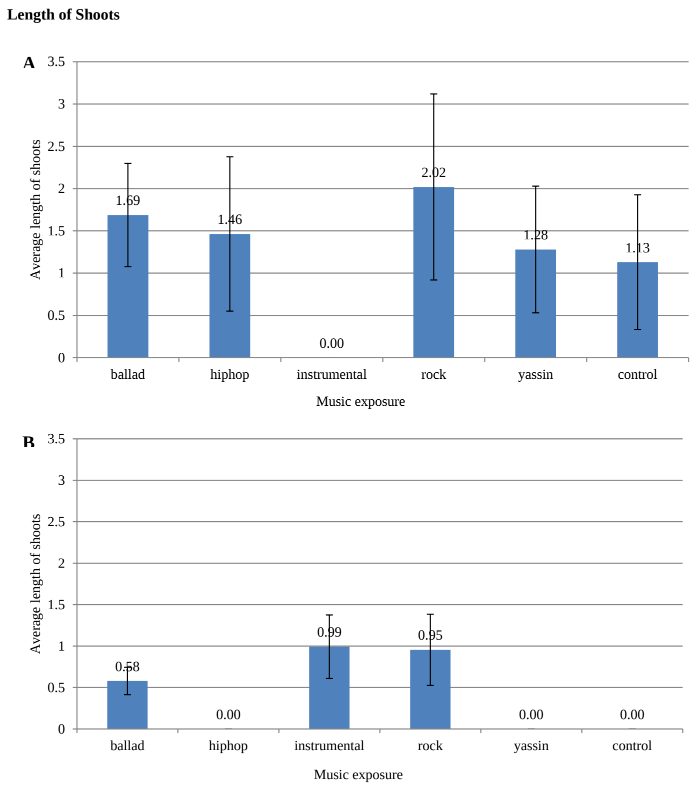

Fig. (6). Effects of different music exposures on the length of shoots developed for *G. hybrid* (**A**) and *G. stapeliiflorum* (**B**). Graphs were plotted based on the measured shoot lengths in the final month of the study. All results were expressed as mean ± SD.

CONCLUSION

Music gave a positive effect on the *in vitro* seed germination and plant growth rate. Different genre of music exposure resulted in different growth rates on certain orchid species. Based on the findings, it can be concluded that different types of orchids need different types of music to influence the rate of its germination and growth. The achievement in discovering the best music to enhance orchid grow can promote a mass production of orchids worldwide. Further research is needed to test different orchid species with different types of music and to elucidate the mechanism of audio frequency affecting the growth rate of orchids.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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