Anubias (Anubias sp.) propagation trough hydroponic culture technique

L Sholichah, M Yamin, R Ginanjar and N Meilisza
Research Institute for Ornamental Fish Culture
Perikanan Street #13, Pancoranmas, Depok
West Java, Indonesia
lilisholichah@kkp.go.id

Abstract. Anubias, water ornamental plants (Anubias sp) are exotic water ornamental plants from Africa that have a high economic value. Apart from its potential economic value, there are still many obstacles faced in the process of aquaculture. One of them is related to the propagation and slow growth. This research was conducted to find the most suitable planting media for the propagation and growth of Anubias through hydroponic techniques. The hydroponic culture was carried out with four treatments in the form of planting media which consisted of: (1) husk charcoal; (2) sand (pasir Malang); (3) gravel; (4) Rockwool; all treatments were carried out by floating / NFT (nutrient film technic) technique. The design used in this study was a completely randomized design, where each treatment was given three replications. Parameters observed and measured included root length, plant height, number of leaves and number of new shoots. The sand as a planting media provides better growth compared to the other three growing media.

1. Introduction
The use of aquatic plants, particularly ornamental plants, for aquarium continues to grow each year. It is reflected by the increasing public interest in designing their room with aquascape. This ornamental plant has high attractiveness due to its exotic and decorative appearance. Thus many people are interested in choosing this plant as one of important components of park. Lately, aquatic plants are not separated from ornamental fish business industry. Statistical data recorded that export value of ornamental fish and aquatic plants from Indonesia reached Rp. 1.7 trillion [1]. This shows that aquatic plants have the possibility to be one of the new main sectors to support ornamental fish export.

According to [2] and [3] aquatic plant is classified to 4 (four) types, namely Oxygenator, Bog Plant, Marginal Plant, and Floating Plant. Among many aquatic plants, there are two species that are relatively expensive and in great demand, those are Bucephalandra sp and Anubias sp. Despite having quite high economic value, both plants are considered slow-growing species compared to other aquatic plants [4] [5]. Anubias is aquatic plant originates from West Africa. Since 2012, study on this plant cultivation has been conducted in the Research Institute for Ornamental Fish, Depok (Balai Riset Budidaya Ikan Hias, Depok). The hydroponic found during Anubias sp. planting maintenance was the symptom of leaf burn and damage when the plant was moved outdoor, even though 70% of plants were covered by parnet. It is expected that Anubias sp leaves are less adaptive to different light intensity, temperature, and humidity outside [6].
In addition to its poor adaptability, other problems commonly occur when cultivating these plants include plant propagation technique that is not yet acquired and slow growth of the plant. Several results of study showed that effort to propagate Anubias plant had been made through methods like micropropagation [7] and invitro propagation [8]. Moreover, propagation effort by conventional methods has not been widely applied. Therefore, various efforts are necessary to ensure successful plant cultivation. One possible alternative to apply is plant cultivation by hydroponics.

The hydroponic culture technique is plant cultivation that does not require soil as planting media or known as “Soil-less Culture” [9]. The term comes from the Greek words; Hydro means water and Pono means working. Thus hydroponics means water and working [10]. Hydroponics is agricultural activity by using water as growing medium to replace soil [11]. [12] mentioned that hydroponics system distributes nutrients to plant roots through irrigation systems in the form of recirculation or stagnant water system where plant root is submerged in soil free-media (in the water). Besides plant propagation purposes, hydroponics technique also has a function as phytofiltration to filter harmful substances in the water. Some previous findings showed that phytofiltration technique was found to be quite effective in reducing heavy metal content such as uranium, lead, and cesium [13] also iron [14] in contaminated water.

Hydroponics is categorized into two methods. Those are substrate hydroponics and NFT (Nutrient Film Technique). However, any new technological development from the two basic methods can be done by adjusting to the room or space and financial condition [11]. Hydroponics culture system has been widely conducted for quite a long time and mostly applied for terrestrial plants such as vegetables and flowering plants. However, hydroponics technic for ornamental water plants, particularly Anubias is rarely performed. Hence, applying this technique is considered new in Anubias cultivation.

Correct growing media will also affect plant growth, added to hydroponics technique. The use of growing media in hydroponics system is called substrate hydroponics. According to [15], substrate hydroponics system is planted cultivation method using solid media in which root will grow in porous substrate given nutrient solution, thus enables plants to obtain enough water, nutrient, and oxygen. [16] mentioned that substrate and nutrient significantly affected the number of chili leaves. The use of different organic and inorganic substrates affected nutrient absorption in plants, maximum growth, optimal water intake, oxygen availability [17].

Based on those conditions, it is necessary to conduct the study in order to observe the extent of different growing media and hydroponics culture systems that may provide interaction or effect on the propagation and grow of Anubias plant.

2. Material and methods
The material used in this study was Anubias sp plant, i.e. Anubias barteri var nana. The plant was obtained from Anubias farmer in Sentul, Bogor West Java. Before treatment, plant was adapted to the climate condition in the experimental unit for a week.

After a week of the adaptation process, Anubias plant with average weight of 1.2 g and average height of 6.2 cm were placed in seedling containers (1 plant per container). The research was carried out in the Greenhouse Laboratory of the Research Institute for Ornamental Fish, Depok (BRBIH). Treatment applied was different growing media that would be used in hydroponics culture technique, consisted of (1) husk charcoal growing media; (2) Malang sand growing media; (3) gravel growing media; and (4) Rockwool growing media.

Moreover, the experimental stage was conducted through a series of activities, as described below.

2.1 Seed sowing
The seed of the Anubias plant is in the form of buds growing on the rhizome stem. Buds were further sown in the sowing tray. The seeds selected had germination rate above 80%. Sowing media commonly used is Rockwool since it can be applied practically due to high water absorption and
sterile property. Seeds that are old enough, marked by the emergence of plumule and radicle, were moved to the growing media.

2.2 Preparation of Growing Media
Growing media applied in this study consisted of four types according to the treatment: husk charcoal, Malang sand, gravel, and Rockwool. Growing media with high porosity was selected to ensure nutrient requirements for Anubias plant. Growing media was placed in the desired container such as clay pot or plastic pot with holes in the bottom and side of the pot to allow for water and nutrient entry.

2.3 Provision of Nutrients
Appropriate hydroponics nutrient was applied since the nutrient provision in hydroponics is really important for plant growth. The need for macro element was able to be met through the addition of 6 g of urea, 9 g of SP, 2 g of K, 5 g of Epsom salt, and 7.5 g of calcium carbonate. Moreover, microelement was able to be fulfilled by adding 2.86 g of boric acid, 0.22 g of sulfuric acid, 2.03 g of manganese sulfate, 0.08 g of copper (II) sulfate, 0.02 g of molybdic acid, and 7.5 g of Fe-chelate. The application was similar to how NPK is applied, namely by dissolving all macro and microelements into 10 liters of water.

2.4 Maintenance
Basically, maintenance in the hydroponics system and conventional system is quite alike, involving pruning, weeding, etc. Observation on plant conditions was performed daily, while growth sampling was only conducted in the beginning and at the end of study. The sampling duration was adjusted to the slow growth of Anubias plant. Parameters observed during study included:
- The number of leaves
- The condition of leaves
- The grow of new buds
- Plant height
- Root length

2.5 Data Analysis
All parameters were analyzed using descriptive analysis.

3. Result and discussion
Anubias sp ornamental aquatic plant is maintained in an emersed system (half part of the plant is immersed in the water, while the other half is directly exposed to the air). This semi-terrestrial maintenance is mostly applied by aquatic plant hobbyists since it is more efficient than immersing the aquatic plant completely in the water. Carbon dioxide or CO\text{2} required by plants are able to be directly obtained from the air without CO\text{2} injection. However, application of this maintenance method is considered insufficient to support the growth of Anubias sp plant optimally. Therefore, it is necessary to conduct some environmental manipulations to ensure plant growth and development. One manipulation effort is applying hydroponics system in plant maintenance. To what extent do hydroponics systems and different growing media affect the growth of Anubias plant is presented in this result of study.

3.1 Quantitative and qualitative performance of leaves
The leaf is the most important and attractive part of Anubias plant. Its thick and wide shape of leaf makes this plant to be suitable for decoration in aquarium with plants. The price of this plant is also affected by the shape, performance, and number of leaves. More solid shape and larger number of leaves will result in higher price of Anubias plant. The result of observation on leaf condition (number and condition) during study is depicted in Figures 1 and 2.
3.1.1 Leaves number. Figure 1 shows a description of the increasing number of leaves during plant maintenance. The number of leaves in each plant at the beginning of maintenance was similar, namely 6 leaves. However, at the end of research, growing media of Malang sand produced a number of new leaves amounted to 9 leaves, followed respectively by husk charcoal media of 8 leaves, Rockwool media of 6 leaves (unchanged), and gravel media with no leaves at all.

3.1.2 Leaves condition. Furthermore, the condition of leaves at the end of maintenance is illustrated in Figure 2 and Figure 3. After maintenance, there was a quite significant change in which leaves were of worse condition when compared to the initial appearance. The quantification method of qualitative data is required to get a quantitative description regarding visual observation. Therefore, scoring method is made to obtain information about leaf condition, from the best to the worst condition. Scoring format of leaf condition is defined as follow:

0 = dead
1 = leaf is almost dead
2 = leaf is drying, torn, and yellow
3 = leaf has small holes, lighter green color
4 = leaf is perfect, has a darker green color, no holes/torn leaf is found
The condition of Anubias leaves during the maintenance period is quantified based on the results of visual observations of the leaves before (A) and after maintenance (B).

The graph illustrated in Figure 2 indicates that the average leaf condition at the beginning of maintenance was excellent (score 4) as seen in the picture of Anubias before maintenance (Figure 3A). Anubias leaves had darker green color with high chlorophyll content and perfect leaf condition; leaves were big and tended to be round in shape. However, leaf condition slowly showed a decreasing quality over maintenance period (Figure 3B). Leaves started to turn yellowish, were smaller in size, and had elongated shape. Normally, leaves will fall off after this symptom starts. Another result that yellowish-green color is not caused by the intensity of sunlight that is too high or nutrient deficiencies, but it is caused by a nematode infection [18].

The result of quantification study on leaf condition as depicted in the graph showed that Anubias plant grown in the sand growing media tended to maintain its leaf condition at its best potential. Leaves had darker green color, perfect shape and no hole or torn leaves were found (score 4). Later, the leaves of Anubias in Rockwool media did not show good condition since leaves had tiny holes and lighter green color (score 3). Moreover, leaf condition in husk charcoal media was quite bad as leaves were drying, torn, and had yellow color (score 2). The worst condition was found in leaves of Anubias plant grown in gravel media where leaves suffered from nutrient deficiency, which resulted in plant death.

The leaf has a function in photosynthesis process. According to [18], photosynthesis process in plants occurs by converting light energy and chemical energy through the role of chlorophyll. The number and condition of leaves are closely related to the nutrient required by plant. In the absence of chlorophyll due to nutrient deficiency, plant cannot grow and develop normally. Nutrient deficiency in plant will lead to slower and less maximal growth.

In this study, the type of growing media or substrate used affected the growth of the Anubias plant, in which plants grown in Malang sand media produced better number and condition of leaves compared to other growing media. [16] mentioned that substrate and nutrient significantly affected the number of chili leaves. The use of different organic and inorganic substrate may affect nutrient absorption in plants, maximum growth, optimal water intake, and oxygen availability [17].

3.2 Plant development and the growth of Anubias plant buds

3.2.1 The Grow of New Buds. Anubias plant is composed of several small leaves and a small hard stem called rhizome. According to [20], the rhizome is the extension of horizontal stem that grows underground and may produce buds and new roots. In Anubias plant, rhizome is the most important part of the plant since it functions as a place to store starches and protein. Buds will grow from rhizomes and later develop into new Anubias plants. [8] mentioned that vegetative propagation process of Anubias plant is conducted by separating buds from parent rhizome. The effect of treatment of different growing media on the growth of Anubias’ new buds can be seen in Figure 4.
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Figure 4. The growing of new buds at initial and final maintenance

Based on the graph, it was known that there was only one bud in each plant at the beginning of maintenance. Later, the number of buds increased or remained the same over time. Plant in Malang sand and husk charcoal media produced one more bud. Thus there were two buds of each at the end of maintenance. However, no more bud has grown in the Rockwool media. Hence there was only one bud at the end of study. This finding is different from the study performed by [21] who found that Rockwool media generated the best result regarding plant growth compared to other treatments. Moreover, no bud was found in gravel media. In fact, plant was found dead.

Plant slower growth that resulted in plant dead is expectedly caused by nutrient deficiency. According to [16], the formation of new branches in the chili plant was highly affected by nutrient availability. An adequate amount of nutrients will lead to better plant growth. In this study, plants failed to grow, particularly those grown in the gravel and Rockwool media. It is expected to be strongly related to the ability of plant to absorb nutrient. The inability of plant to absorb nutrients will eventually slow metabolism process that later brings an impact on failure in the formation of new productive branches. [22] mentioned that productive branch is branch that is able to produce flowers and fruits. The absence of new buds in Rockwool and gravel media shows that plants experienced metabolic disorder/malfunction.

3.2.2. Plant height. Plant height was measured from the root neck to the leaf tip, not including the root length. The study results showed different plant height among treatments. Figure 5 shows the development of Anubias plant height until the end of maintenance period. The highest value (in cm) was obtained by treatment of Malang sand media, followed by husk charcoal media treatment and Rockwool of 9.2, 8, and 6.1, respectively. Unfortunately, plants in gravel media were all dead.
According to [23], growth is an irreversible increase in plant size due to cell enlargement and cell division (size and number). Plants maintained in growing media made of Malang sand, and husk charcoal was able to meet their nutrient requirement compared to those grown in Rockwool and gravel. Thus, Anubias plant in Malang sand media grew higher than those in other media. Plant in husk charcoal media also showed fairly good growth trend. This finding proves that husk charcoal is a good growing media to maintain the growth of Anubias plant. The result of study conducted by [24] showed increasing total weight of fruits grown in husk charcoal media. This result might be related to the ability of husk charcoal to absorb water and maintain nutrient well. [25] mentioned that husk charcoal was able to affect the availability of phosphor that has essential role in photosynthesis process which will further affect the rate of relative growth of plant.

Hydroponic culture models can also affect plant height. According to the results of [26], research that the triangular culture model produced a higher plant height than the rectangular model and was statistically significantly different.

3.2.3 Root length. The root is an important part of the plant that affects growth process and life of a plant. The root is vegetative organs of plants besides stem and leaf. The root type of Anubias plant is fibrous root that penetrates into substrate and firmly anchors to the substrate. Hence, the best way to grow Anubias is by attaching it to woods or rocks. Thus roots will be able to absorb nutrients required optimally. Observation result on root length in each treatment is presented in Figure 6.

Measurement of root length of each treatment showed different results. The longest root was found in Malang sand growing media of 18 cm, followed by husk charcoal media of 12.5 cm, Rockwool of 5.7 cm, and gravel of 0 cm. The high value of root length of Anubias grown in Malang sand media shows that the plant has a high root absorption capacity. This result is in line with [27], who mentioned that a high absorption capacity of root is reflected by high root volume. Moreover, the low value of Anubias root length in other treatments shows that the metabolism process is not only affected by the factor of nutrient availability. It may also be related to the adaptability to other environmental factors such as humidity and temperature. According to [28] humidity influences transpiration process in plants. The transpiration process is slower in higher humidity. Thus physiological condition of plant is stable. On the contrary, rapid rate of transpiration occurs in low humidity and adversely affects plant physiological conditions. This will result in imperfect rooting process and leaf formation. In addition to relative humidity, one factor that influences rooting process is the temperature of media. In relatively high temperatures, rooting process cannot be performed properly. Several studies showed the rate of root development inversely proportional to soil
temperature, in which higher temperature results in lower rate of root growth. This condition was found in the study on *Norway spruce* [29] and *Pisum sativum* L [30].

4. Conclusion

The type of growing media affected the propagation and growth of the *Anubias* plant. It was found that each *Anubias* plant showed different responses to its growing media. Malang sand growing media provided better growth and development of *Anubias* plant compared with the other three growing media (husk charcoal, Rockwool, and gravel). However, it is also noted whether growing media is the only factor that affects *Anubias* growth or there is another environmental factor such as humidity and light intensity that has the possibility to affect the growth of *Anubias* plant. Further study concerning the effect of this factor on *Anubias* plant is therefore necessary.

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