EFFECTS OF DIFFERENT LIGHT INTENSITIES AND GROWTH MEDIA ON GROWTH PERFORMANCE OF FRESHWATER LAWN PENNYWORT (Hydrocotyle sibthorpioides Lam.)

CHUAH TSE-SENG¹, YAP CHEE-HEUNG¹ and ISMAIL B.S.²*

¹Faculty of Agro-technology and Food Science, Kolej Universiti Sains dan Teknologi Malaysia, KUSTEM, 21030, Kuala Terengganu, Terengganu, Malaysia

²School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor, Malaysia
E-mail: ismail@pkrisc.cc.ukm.my

ABSTRACT
The aim of this study was to determine the interaction between growth medium and light intensity on the growth of the freshwater aquatic plant, Hydrocotyle sibthorpioides under glasshouse conditions. The plants were grown with two growth media, namely Hoagland nutrient solution with or without sand, at three different light levels, viz 100% (800 – 1200 μEm⁻²s⁻¹), 60% (480 – 720 μEm⁻²s⁻¹) and 40% (320 – 480 μEm⁻²s⁻¹). Generally, light could stimulate the growth of Hydrocotyle sibthorpioides when the light level was increased from 60% to 100% regardless of growth media. However, no significant difference was found in all growth parameters when light level was increased from 40% to 60%. Hydrocotyle sibthorpioides exhibited the highest number of new leaves formed, dry weight of total new leaves and leaf surface area at 100% light level under nutrient solution medium without sand, suggesting growth of Hydrocotyle sibthorpioides is optimal when grown under this medium at the 100% light level.

INTRODUCTION
In the wild, Hydrocotyle sibthorpioides Lam. is an amphibious softwater plant that commonly grows in dry, moist and wet locations (Kasselmann 2003). This umbelliferous plant has a strong tendency to float along the water surface and occasionally is found entirely submerged (Jacobsen 1979). It is commercially available due to its attractive leaf morphology that wins its placement in the midground to foreground position or at the front corners of aquarium. However, Hydrocotyle sibthorpioides is an aquarium plant which is hard to cultivate (Anon 2002). Although it has been documented that this plant can grow either under shaded, shady-sunny or full sunlight
areas based on field observation (Kasselmann 2003), such limited information on the plant’s light requirement is clearly inadequate as basis for successful cultivation. An understanding of the response of *H. sibthorpioides* to different light levels under different growth media is valuable for maintenance and cultivation of this aquarium plant. Hence, this study was conducted to examine the effects of different light intensities on the growth performance of *H. sibthorpioides* under two growth media, that is, nutrient solution with or without sand.

**MATERIALS AND METHODS**

*Plant Materials*

Wild seedlings of *H. sibthorpioides* were collected from a nursery in Gombadak, Kuala Terrenganu. The plants were propagated in trays (35 x 25 x 10 cm) under glasshouse conditions with temperature and light intensity ranging from 29 – 32°C and 800 – 1200 μEm⁻²s⁻¹, respectively.

**Greenhouse experiment**

Each runner (1.2 cm leaf length) was transplanted into a bucket containing 300 mL full strength Hoagland nutrient solution (Hoagland and Arnon 1950) with sand (HS) or without sand (HO). The sand was sieved through a nylon net (mesh size: 2.0 mm) and washed, dried and autoclaved at 121°C for 20 minutes prior to use. Buckets containing runners were then divided into three groups. The first group received full sunlight (100% light intensity, 800 – 1200 μEm⁻²s⁻¹), while the other two groups were placed under green netting (25 cm x 25 cm) providing 60% (480 – 720 μEm⁻²s⁻¹) and 40% (320 – 480 μEm⁻²s⁻¹) light intensity. A light meter (LI-COR, Model LI-250A) was used to measure the light intensity. The netting was placed on the top of each bucket.

One or two drops of calcium carbonate (0.2% w/v) or hydrochloric acid (0.5% v/v) were added to stabilize pH of growth media every two days. pH level for both HS or HO medium was maintained at 6.5 – 7.0 and 6.0 – 8.0, respectively.

Distilled water was added to each bucket daily to replace water loss due to evaporation while nutrient solution was given constantly every two weeks until the end of the study. During the experiment, a few drops of algacide (0.05% v/v) were added to both HO and HS medium weekly.

Throughout the experiment, the number of new leaves formed by runner was recorded weekly. After six weeks, each whole plant was harvested and separated into aboveground and underground tissues. Each plant portion was dried in an oven at 65°C for 48 hours. Total dry weight of plant, dry weight of leaves and dry weight of stem plus root were determined. A portable leaf area meter (CID, Model C1-202) was used to measure the leaf surface area.

**Statistical Analysis**

The experiment was laid out in a completely randomized design with five replications. General Linear Model was performed using the MINITAB, statistical software program to compare means across treatments. All data were checked for the satisfaction of normality and equal variance assumptions. Square-root-transformation was performed on leaf surface area data. A post-hoc Tukey’s honestly significant difference test was used to determine differences between treatments at the 5% of significance level.

**RESULTS AND DISCUSSION**

The number of new leaves formed under Hoagland nutrient solution medium with sand (HS) increased gradually as light intensity increased. The number of new leaves was recorded at approximately 2, 3, and 5 days at 40%, 60% and 100% light intensity, respectively. The post-hoc Tukey’s test revealed that there was a significant increase in the number of new leaves formed when the light level was increased either from 40% or 60% to 100% under HS medium (Table 1). When the experiment was terminated after six weeks, the maximum observed number of new leaves formed by *Hydrocotyle sibthorpioides* was approximately 9 at 100% light intensity under Hoagland solution medium without sand (HO) while the number of new leaves at 40% light level was reduced to 2 (Table 1).

There was a significant interaction between growth medium and light level for *H. sibthorpioides* influencing the number of new leaves formed (p<0.0001). When light intensity was 100%, the number of new leaves formed was found to be the highest irrespective of growth media. This result is in agreement with the finding by Hiscock (2003) who reported that *H. sibthorpioides* needs a high-intensity light. Madsen et al. (1991) found that aquatic plants with higher photosynthetic rates could increase their metabolic rate, resulting in increased productivity under high light intensity. However, plants grown in HO medium exhibited a greater number of new leaves (p<0.05) than did plants grown in HS medium at 100% light level.

In the HS medium, dry weight of new leaves increased from 0.0194g to 0.0369g as light level increased from 40% to 100% (Table 1), indicating that light intensity has a positive effect on dry
weight of total new leaves as light intensity increased from 40% to 100%. Similarly, a noticeable increment occurred when light level was increased from 40% to 100% for plants grown in the HO medium.

Analysis of variance (ANOVA) indicated that the interaction between growth medium and light intensity was significant (p=0.007). Different responses in terms of dry weight of total new leaves under both growth media were observed at light levels of 40% and 100%. At the 100% light level, biomass production in terms of total new leaves formed was higher when plants were grown in the HO medium than when grown in HS medium. When the light intensity was 40%, dry weight of total new leaves was reduced in both growth media as the post-hoc Tukey’s test revealed. However, there was no significant difference in dry weight of total new leaves between light intensities of 60% and 40% regardless of growth media.

Under full sunlight, photosynthesis of *H. sibthorpioides* was maximal, thereby resulting in greater biomass of new leaves under both growth media. Under low light intensity, *H. sibthorpioides* exhibited low dry weight of total new leaves under both growth media. This may correspond to a study by Patterson (1980) who showed that the reduction in dry matter production under shading was due to significant reductions in net assimilation rate or total leaf surface area.

The post-hoc Tukey’s test indicated that there was no significant difference in total leaf surface area between plants grown at light levels of 40% and 100% in the HS medium. Conversely, total leaf surface area differed significantly as light intensity increased from 40 to 100% in the HO medium, with leaf surface area being the highest under 100% light intensity as compared to total leaf surface area exhibited in HS medium (Table 1).

ANOVA showed that effects of the interaction between growth medium and light intensity on total leaf surface area were significantly different (p<0.05). Total leaf surface area of plants grown in HO medium was much higher than those plants grown in HS medium, giving about 5 cm² of leaf surface area at 100% light level in HO medium.

Increasing total leaf surface area of *H. sibthorpioides* captures more solar energy to synthesize food by photosynthesis. Ridge (1987) has reported that increased leaf extension in response to submergence is a common response of many aquatic plants, mediated by the release of ethylene. Based on the results of the present study, an increase in leaf surface area of *H. sibthorpioides* may be due to greater numbers of new submerged leaves formed under HO medium at 100% light level (Table 1). In contrast, new leaves that emerged under HS medium were retarded, thus producing less leaf surface area at 100% light level.

Effects of light intensity on dry weight of stem plus root and total dry weight of plant are shown in Table 2. ANOVA revealed that there was no significant effect of interaction between growth medium and light intensity on dry weight of stem plus root (p=0.480) nor on total dry weight of plants (p=0.081). However, both stem plus root dry weight and total dry weight of plant exhibited positive responses towards the high-intensity light at 100%.

Irrespective of the growth medium factor, the dry weight of stem plus root increased with increasing light levels. A post-hoc Tukey’s test

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**Table 1.** Effects of different light intensities and growth media on number of new leaves formed, dry weight of total new leaves and leaf surface area at 6th week

<table>
<thead>
<tr>
<th>Types of Growth Media</th>
<th>Hoagland solution with sand (HS)</th>
<th>Hoagland solution without sand (HO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light intensity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>No. of new leaves/ plant</td>
<td>2.00±0.00* a</td>
<td>3.33±0.30* a</td>
</tr>
<tr>
<td></td>
<td>5.33±0.33* b</td>
<td>2.00±0.00* a</td>
</tr>
<tr>
<td></td>
<td>5.33±0.33* b</td>
<td>2.00±0.00* a</td>
</tr>
<tr>
<td></td>
<td>8.50±0.50* c</td>
<td>8.50±0.50* c</td>
</tr>
<tr>
<td>Dry weight of total new leaves (g/plant)</td>
<td>0.0194±0.0013* a</td>
<td>0.0294±0.0025ab</td>
</tr>
<tr>
<td></td>
<td>0.0369±0.0053b</td>
<td>0.0221±0.0010ab</td>
</tr>
<tr>
<td></td>
<td>0.0189±0.0037a</td>
<td>0.0553±0.0007c</td>
</tr>
<tr>
<td>Leaf surface area (cm²/plant)</td>
<td>0.40±0.05* a</td>
<td>1.24±0.39* a</td>
</tr>
<tr>
<td></td>
<td>1.43±0.36* a</td>
<td>0.61±0.15* a</td>
</tr>
<tr>
<td></td>
<td>1.50±0.33* a</td>
<td>4.85±0.90* a</td>
</tr>
</tbody>
</table>

* denotes means and standard errors. Identical letters within the same row indicate that data are not significantly different (p>0.05) between treatments as determined by Tukey’s test.
Table 2. Effects of different light intensities on dry weight of stem plus root and total dry weight of plant at 6th week

<table>
<thead>
<tr>
<th>Light intensity (%)</th>
<th>Dry weight of stem plus root (g/plant)</th>
<th>Total dry weight of plant (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.0291 ± 0.0070**</td>
<td>0.0496 ± 0.0074*</td>
</tr>
<tr>
<td>60</td>
<td>0.0411 ± 0.0079*</td>
<td>0.0676 ± 0.0093*</td>
</tr>
<tr>
<td>100</td>
<td>0.0822 ± 0.0078*</td>
<td>0.1311 ± 0.0118*</td>
</tr>
</tbody>
</table>

* denotes means and standard errors. Identical letters within the same column indicate that the data are not significantly different (p>0.05) between treatments as determined by Tukey’s test.

The results of this study revealed that Hydrocotyle sibthorpioides is an amphibious plant that could adapt to both HO and HS media with the influence of different light intensities. Conversely, a previous study by Chambers & Kalff (1985) showed that sediment composition was more important than irradiance in controlling the growth of Myriophyllum spicatum. Mohammad (2005) reported that partitioning of photosynthetic products is crucially important to the reproductive growth. A sustained supply of photoassimilates is needed for flowering, fruit and seed set. It was observed that inflorescence of H. sibthorpioides formed at 60% and 100% light levels (data not shown) under HO medium.

Mohammad (2005) pointed out that during photosynthesis, photoassimilate partitioning is under the control of two component systems namely source production and sink. Roots, stems and young leaves are competitive sinks for assimilates at the vegetative stage (Mohammad 2005). Similarly, the present result indicates that more photoassimilates were produced by the parent plant of H. sibthorpioides (source) during photosynthesis, while more new leaves and stems plus roots were formed as a sink. Thus, H. sibthorpioides had greater biomass production in stem plus root and total amount of new leaves formed at 100% light level than did plants grown under 40% and 60% light levels (Tables 1 & 2).

Kasselman (2005) reported that leaves of aquatic plants are able to absorb oxygen, carbon dioxide and nutrients directly from the water. For instance, water lily, Brasenia schreberi, a softwater plant, displays countless hydropotes on the leaf’s underside which assist in the absorption of water and mineral nutrients. This may explain the increase in new leaves formed, in dry weight of total new leaves and in leaf surface area of H. sibthorpioides when grown in HO medium than when grown in HS medium. Hydrocotyle sibthorpioides may have an extraordinary characteristic and ability to absorb nutrients and water through its floating leaf surfaces. As a result, good adaptation of leaves of H. sibthorpioides under HO medium have optimized its growth and development at 100% light level.

In HS medium, H. sibthorpioides exhibited fewer of new leaves formed, lower dry weight of total new leaves and lower leaf surface area than when it was grown in HO medium at 100% light level. This is probably due to the edaphic characteristics of the growth medium. Walstad (2003) pointed out that almost all sediments supporting aquatic plant growth are anaerobic. Under these anaerobic conditions, water-saturated sediments could pose several problems to aquatic plants, including low redox and toxicity from...
heavy metals, hydrogen sulfide and organic acids. Hence, the HS medium was not as conducive to the growth of *H. sibthorpioides* as the HO medium.

Furthermore, Walstad (2003) reported that root oxygen release is critical for aquatic plant survival in anaerobic substrates. Waterlogged organic substrates, usually devoid of oxygen, may contain reduced phytotoxic substances like ferrous iron and methane (Crawford, 1983). Plants generally do not tolerate under such conditions because below-ground parts need oxygen for respiration. The fact that the floating plant *H. sibthorpioides* thrives in HO medium seems to indicate that it has a well-developed rooting system with root oxygen release to absorb essential water and nutrient when grown under HO medium.

No significant difference was observed between 60% and 40% light levels in all growth parameters measured such as numbers of new leaves, dry weight of total new leaves formed, total leaf surface area, dry weight of stem plus root and total dry weight (Tables 1 and 2). This suggests that insufficient light is a limiting factor for growth of *H. sibthorpioides* at 320 to 720 μEm⁻²s⁻¹.

**CONCLUSION**

Light intensities proved to increase stem, root and total biomass of *Hydrocotyle sibthorpioides* when increased from 60% (480 – 720 μEm⁻²s⁻¹) to 100% (800 – 1200 μEm⁻²s⁻¹), regardless of growth medium. However, the greatest number of new leaves formed, dry weight of total new leaves and total leaf area of *H. sibthorpioides* were recorded under full sunlight of 800 – 1200 μEm⁻²s⁻¹ when plants were grown under nutrient solution medium without sand. The results of this study suggest that a high-light intensity of 800 – 1200 μEm⁻²s⁻¹ is optimal for the growth of *H. sibthorpioides* under nutrient solution medium.

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**REFERENCES**


