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Growth performance of Malaysian's spoongrass, *Halophila ovalis* (R.Br.) Hooker *f.* under different substrate, salinity and light regime

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Abstract—*Halophila ovalis* plants collected from the native environment (Lat. 04°54.473′N, Long. 115°22.299′E-Pantai Bangat, Lawas, Sarawak, Malaysia) were grown in aquarium culture system to assess: (i) the feasibility of using planting materials e.g. rhizomes with and devoid of leaves, (ii) the growth and development of the plants under non-native substrates, fine beach sand and coarse river sand, (iii) the sustain growth and development of the population, and (iv) the tolerance range of plants under the tested salinity and depth. These assessment were achieved by planting rhizomes with or devoid of leaves in the tested substrates in containers, maintained in artificial seawater of various salinity, with minimum aeration and exposed in shaded outdoor natural condition. The artificial seawater permitted a standardization of the medium. By manipulating the placement of substrates in the container (and planting of rhizomes into individual container) then submerged at different depths into the aquarium facilitated observation, recording of data and transferring of plants from one test condition to another. The morphological changes of the vegetative parts e.g. leaf, petiole dimensions and paired cross-veins numbers of *H. ovalis* in the created conditions were compared to those of *H. ovalis* from native environment.

Key words: *Halophila ovalis*, laboratory culture, substrate, tolerance, salinity, depth

Introduction

The attempt of growing seagrass had been conducted as early as 1959 (Wood 1959). Various methods had been employed on various species of seagrasses in order to create a small-scale marine system that simulates the environmental conditions of the natural habitats accordingly to species and locality. Among the successful one is for example at Austin, Texas where representatives of 9 out of 12 genera of seagrasses; *Thalassia*, *Halodule*, *Halophila*, *Posidonia*, *Zostera*, *Cymodocea*, *Syringodium*, *Enhalus* and *Thalassodendron* have been successfully cultured in synthetic seawater and under controlled environmental conditions (McMillan et al. 1981). Using the culture system, seagrasses were maintained that facilitated studies involving the biological, ecological and phenological aspects of seagrasses (McMillan et al. 1981). Although such culture has been developed they were only suitable for a particular type of seagrasses and their conditions of growth under studied. In addition, the origin, biology, distribution and the habitat of seagrasses used in the studies were not similar. For example, *H. ovalis* (R.Br.) Hook *f.* that live in estuaries can tolerate lower salinities than that of deep sea *H. ovalis* because they have to adapt to low salinities caused by freshwater runoff and wet monsoon season (Benjamin et al. 1999). There are no available best methods for culturing the seagrasses (Fuss and Kelly 1969) especially for tropical species. In Malaysia, there are 15 species of seagrasses of which 6 species belong to *Halophila* and *H. ovalis*, being the most common and frequently occurred along the shallow inter-tidal coast. Although common its biology and phenology have rarely been examined (Japar Sidik et al. 2008). The aim of this present study is to assess the growth and development of Malaysian *H. ovalis* using a culture system and techniques that could provide conditions favorable to the plants.

Materials and Methods

A culture system for growing *H. ovalis* comprises 0.45 m×0.45 m×1.22 m glass aquarium, flooded with artificial seawater prepared from commercial sea salt (Marinemix, Marine Enterprises International Inc., Baltimore, USA). The Marinemix is a formulation containing all essential major, minor and trace elements for sustaining the growth and development of *H. ovalis*. The culture system is fitted with an external filter system and a submersible pump to provide filtration and circulation of water inside the aquarium (Fig. 1). Rhizome axes with leaves of *H. ovalis* collected from Pantai Bangat (Lat. 04°54.473′N, Long. 115°22.299′E), Lawas, Sarawak were planted in a substrate placed in a 33 cm×26 cm×9 cm plastic tray. Using these techniques and in the culture system setup (Fig. 1), by manipulating the planting materials (e.g. using rhizome axes devoid of leaves), placement of non-native substrates of different textures and sources (fine beach sand taken from Tanjung Batu beach, Bintulu; coarse river sand from a small stream at Universiti Putra Malaysia Bintulu Sarawak Campus) in the plastic tray (i.e. the planting of rhizomes into individual container) then submerged in the separate aquaria containing water of different salinity (25, 30 and 40 psu) and depth (10, 20, 30 and 40 cm

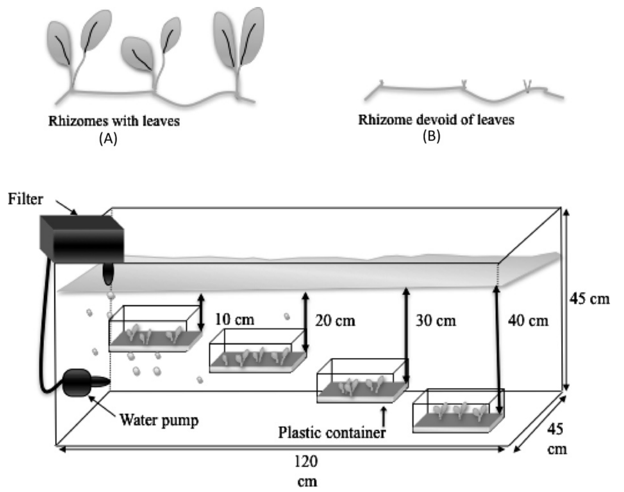

Fig. 1. The planting materials, rhizome axes with leaves (A) or rhizome axes devoid of leaves (B) are planted in a substrate in plastic containers, immersed at different depths in a culture system (C) containing artificial seawater.

from the water surface) facilitates observation, recording of morphological data, tolerance and adaptation of plants from one test condition to another. The culture system was kept under a shaded out-door natural light regimes that fluctuated from 160 μmol/m^2/sec (10% of the ambient light intensity outside) to ~240 μmol/m^2/sec (15% of the ambient light intensity outside) recorded between 12.00 noon to 2.00 pm. Water temperature and pH were recorded as 24–29°C and 8.0–8.5, respectively. Water level height of 40 cm (the water salinity maintained) in each of the aquarium was maintained by adding distilled water. To maintain the water clarity, the external filter was cleaned with distilled water bimonthly. After six months, 25% by volume of the water was replaced to replenish the nutrients and to improve the water quality of the aquarium. The indicators for the growth performance and development were the morphological changes of the vegetative parts formed e.g. leaves, rhizomes in adapting to the created conditions as compared to the native environment, sustain growth and the development of the population, reproductive biology (flowering, anthesis and fruiting) and the pattern of seedling development from seeds to mature plants. The data were analyzed to test the significant variation in leaf width, length, petiole length, number of cross-veins for *H. ovalis* for each treatment using ANOVA and if significant differences were detected, this was followed by Duncan's Multiple Range test (SPSS for Windows Release 10.0).

Results and Discussion

The feasibility of using different forms of planting materials

Rhizomes with leaves or devoid of leaves (Fig. 1) were successfully grown in the tested substrates maintained in artificial seawater of various salinity and water depth, with minimum aeration exposed in shaded out-door natural condition. Rhizomes devoid of leaves were never used in any of seagrass culture studies. This offers an advantage where rhizomes devoid of leaves can be sampled without having to

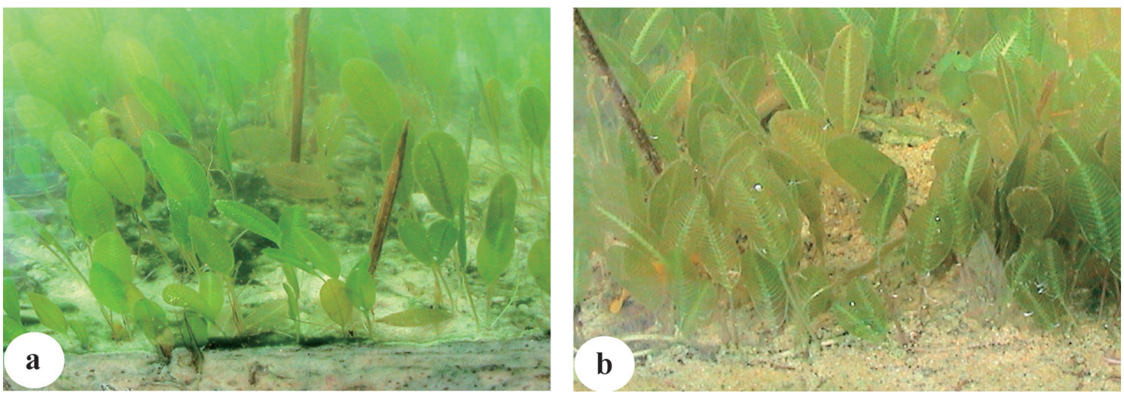

Fig. 2. *Halophila ovalis* propagated from rhizome axes devoid of leaves in (a) fine beach sand (b) coarse river sand.

collect masses of plants together with substrates employed in other studies where the planting materials were sampled as cores (Prange and Dennison 2000), sprigs (Kenworthy and Fonseca 1977, Hillman et al. 1995, Benjamin et al. 1999), or plugs (McMillan and Moseley 1967, Abal et al. 1994, Longstaff et al. 1999). However, Moffler and Durako (1984) used seeds in the culture study of *Thalassia testudinum*.

The growth and development of the plants under non-native substrates

Plants grew and developed equally well in the fine beach sand (Fig. 2a) and coarse river sand (Fig. 2b). Comparatively based on leaf sizes, plants showed better growth and development in coarse river sand (Table 1 compare with Table 2, Fig. 3 for plants from the wild). *Halophila ovalis* from the native environment can be categorized into three morphological forms (small-, intermediate- and big-leaved). They were found at similar depth of 1.5 m but growing on different substrates, i.e. muddy, sandy, and sandy-mud substrates respectively (Fig. 3). Den Hartog (1970) reported that *H. ovalis* was found to occur on all kinds of substrate ranging from coarse rubble to soft mud. *Halophila ovalis* exhibited an array of leaf morphology with respect to sizes and shapes and has been attributed to substrate (Young and Kirkman 1975) and also environmental factors such as salinity (Benjamin et al. 1999) and shade (Japar Sidik et al. 2001).

The tolerance range of plants under the different salinity and depth

Halophila plants have no problem in adapting to higher salinities compared to the ambient salinity of 29 psu in the native habitat. Better growth performance was observed at salinity 25 psu (Table 1). *Halophila ovalis* is rather euryhaline and penetrates water with an average salinity and even less saline waters into estuaries and sea-inlets and is not uncommon in the brackish coastal lagoons. On the island of Oahu (Hawaii), the species has been found under hyperhaline conditions according to den Hartog (1970). Distribution and

Table 1. *Halophila ovalis* propagated from rhizomes devoid of leaves, exposed to different treatments in shaded out-door natural conditions. Morphological variations as demonstrated by the leaf size and cross veins number after eight weeks of culture. Means with the same letter in rows are not significant different at $p<0.05$ by Duncan Multiple Range Test.

Parameters			Leaf width (mm)	Leaf length (mm)	Leaf petiole (mm)	Cross veins (no.)
			Mean±s.d (Range) N	Mean±s.d (Range) N	Mean±s.d (Range) N	Mean±s.d (Range) N
Halophila ovalis in culture	Substrate Water salinity 30 psu	Coarse river sand	11.00 ± 1.67^{a} (7.23–14.55) 72	26.06 ± 5.52^{a} (16.79–47.53) 72	29.92 ± 7.30^{a} (16.10–45.92) 72	15.58 ± 2.09^{a} (11–20) 72
		Fine beach sand	6.88 ± 2.59^{b} (3–11.86) 72	15.15 ± 5.22^{b} (6.72–25.98) 72	17.16 ± 7.28^{b} (8.12–38.69) 72	11.56 ± 1.88^{b} (5–17) 72
	Coarse river sand Water salinity (psu)	25	11.27 ± 1.55^{a} (8.76–14.45) 48	26.69 ± 6.52^{a} (16.67–47.53) 48	30.59 ± 7.83^{a} (17.56–45.73) 48	15.04 ± 2.63^{a} (11–20) 48
			7.81 ± 3.42^{b} (3.00–14.55) 48	17.25 ± 6.70^{b} (6.72–27.35) 48	20.74 ± 3.79^{b} (8.29–45.92) 48	12.60 ± 2.66^{b} (5–18) 48
		40	7.76 ± 2.25^{b} (4.36–12.2) 48	17.88 ± 5.83^{b} (10.09–29.21) 48	19.30 ± 7.60^{b} (8.12–36.45) 48	13.08 ± 2.63^{b} (9–18) 48
	Coarse river sand Water depth (cm)	10	8.75 ± 2.62^{b} (4.36–12.94) 36	19.77 ± 6.16^{b} (10.09–32.08) 36	24.84 ± 9.80^{ab} (10.34–42.59) 36	13.03 ± 1.99^{b} (10–17) 36
		20	9.52 ± 3.24^{a} (3.21–14.45) 36	21.65 ± 6.82^{a} (9.59–36.12) 36	25.48 ± 11.33^{a} (9.52–45.92) 36	13.78 ± 2.69^{a} (9–20) 36
		30	8.89 ± 3.38^{b} (3.19–14.55) 36	21.50 ± 10.07^{a} (8.59–47.53) 36	20.76 ± 7.95^{c} (8.12–31.73) 36	13.52 ± 2.87^{ab} (9–19) 36
		40	8.61 ± 2.73^{b} (3.00–11.69) 36	19.51 ± 7.06^{b} (6.72–30.35) 36	23.09 ± 9.05^{b} (9.50–45.73) 36	13.97 ± 3.54^{a} (5–20) 36

Table 2. *Halophila ovalis* from the wild or native habitat. Morphological variations as demonstrated by the leaf size and cross veins number. Means with the same letter in rows are not significant different at p<0.05 by Duncan Multiple Range Test.

Parameters			Leaf width (mm)	Leaf length (mm)	Leaf petiole (mm)	Cross veins (mm)
			Mean±s.d (Range) N	Mean±s.d (Range) N	Mean±s.d (Range) N	Mean±s.d (Range) N
Halophila ovalis from the wild or native habitat	Substrate Water salinity 29 psu	Sandy mud	10.44 ± 1.18^{a} (8.38–12.86) 31	20.57 ± 4.07^{a} (7.91–10.88) 31	29.81 ± 6.67^{a} (4.81–13.02) 72	14.45 ± 2.35^{a} (10–19) 20
		Sandy	8.72 ± 0.73^{b} (7.55–10.13) 31	16.19 ± 2.1^{b} (9.03–10.34) 31	22.27 ± 5.11^{b} (7.74–10.27) 144	11.6 ± 1.81^{b} (8–15) 20
		Muddy	6.72 ± 0.76^{c} (5.63–8.89) 31	12.31 ± 1.15^{c} (4.30–10.38) 31	16.00 ± 6.03^{c} (3.95–34.4) 149	10.65 ± 1.87^{b} (7–15) 20

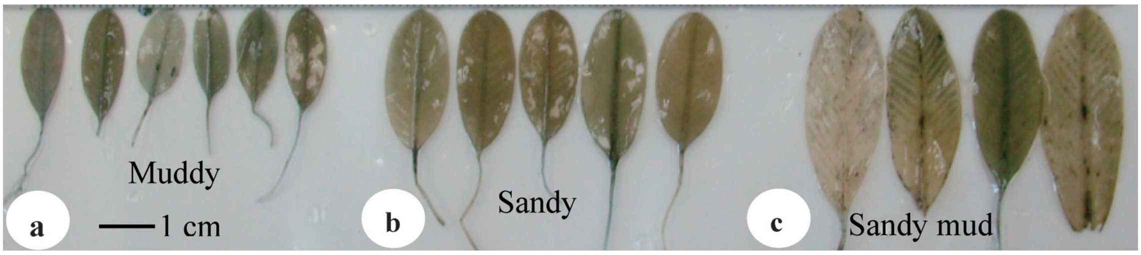

Fig. 3. Variation in leaf morphology of *Halophila ovalis* from the native habitat categorized as (a) small-leaved from muddy substrate, (b) intermediate-leaved from sandy substrate and (c) big-leaved from sandy mud substrate.

abundance of seagrasses in an environment is controlled by range of environmental condition including light availability which is considered as one of the most important environmental parameters, controlling the depth to which seagrasses can grow and excluding seagrasses from areas with low light conditions (Longstaff and Dennison 1999). The culture system was kept under a shaded out-door natural light regimes of 160 μmol/m^2/sec to ~240 μmol/m^2/sec and plants at water depth of 20 cm from surface water level showed better growth performance compared to other depths (Table 1). It is recognized in this experiment that *H. ovalis* possesses tolerance to low light intensity, however, the actual quantity required by the plant has not studied. The average requirement of seagrasses as a group of plant has been calculated to be 11% of surface light by Duarte (1991). Based on this present experimental setup, the water level and hence the volume and quantity of articicial seawater salt can to be reduced to halve. In addition the shallower culture tank could be used for growing of *H. ovalis*.

Production of reproductive materials

Plants under the varying conditions, e.g. substrate, salinity and depth were producing flowers and fruits. *Halophila ovalis* is dioecious, i.e. male and female plants are separated. Male flowers lasted for brief period of 2 days, and female flowers lasted about 1 month and flowering is seasonal. This maybe the reason plants collected from the wild usually devoid of flowers. In the field, inconspicuous flowers and fruits have probably been overlooked because of the short lasting of particularly the male flowers and the tiny flowers of the female. In the wild it is also difficult to distinguish between male and female plants as they can only be identified when they are in flowering season. Under the culture system, we can identify, separate and follow the progressive phenology development of male and female *H. ovalis* plants. Few seagrasses produce flowers under culture conditions except for studies by McMillan (1980, 1987). Viable seeds were produced in the culture as demonstrated by the progressive development of seeds to seedlings, juvenile plants and to mature and flowering plants sustained in the system. *Halophila ovalis* produces flowers and fruits, and propagates by vegetative and reproductive means.

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