UNIVERSITI PUTRA MALAYSIA

SHADING RESPONSES OF THE SEAGRASS *HALOPHILA OVALIS* (R. BR.) HOOK. F. FROM TELUK KEMANG, NEGRI SEMBILAN, MALAYSIA

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***HALOPHILA OVALIS* (R. BR.) HOOK. F. FROM**
TELUK KEMANG, NEGRI SEMBILAN, MALAYSIA

By

MOHAMMAD ROZAIMI B JAMALUDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Masters of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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June 2008

Chairman: Japar Sidik Bujang, PhD

Faculty: Science

The seagrass *Halophila ovalis* from Teluk Kemang coast (2 ° 30'N, 101 ° 45'E) in Port Dickson, Negeri Sembilan was studied to elucidate its responses towards artificial shading. Responses were firstly based on autotrophic productivity of *H. ovalis* through photosynthesis experiments to determine the effects of prior acclimation to the condition of either in the field (naturally growing) or in cultures (light reduced to 85-90% of ambient conditions). Results showed that the light compensation values in field and cultured leaves (8-13 µmol m^{-2} s^{-1}) were similar while saturation point was in the range of 268-275 µmol m^{-2} s^{-1} for field leaves and increased to 290-293 µmol m^{-2} s^{-1} for cultured leaves. A one-month long artificially imposed shading was then performed to plants in the field (50%, 65%, 80% and 95% shading relative to field light intensity) and in cultures (92% shading – Tank 1, and 96% shading – Tank 2, relative to field light intensity) and compared to unshaded plants as a control showed the following responses. Photosynthetic rates of field *H. ovalis* at two tide levels as determined using

the Biological Oxygen Demand bottle method was up to six times higher when compared to the oxygen electrode method. Leaf chlorophyll content was significantly higher from plants under shading for both field and cultured leaves compared to control where leaves from cultures (Tank 2) showed the highest value in leaf chlorophyll content (1353.40 $\pm$ 74.00 µg chlorophyll *a* g^{-1}, $p < 0.01$, and 11.92 $\pm$ 0.59 µg chlorophyll *a* cm^{-2}, $p < 0.01$, by leaf fresh weight and leaf surface area respectively, and 744.30 $\pm$ 46.55 chlorophyll *b* g^{-1} , $p < 0.01$ and 6.56 $\pm$ 0.39 µg chlorophyll *b* cm^{-2} , $p < 0.01$, by leaf fresh weight and leaf surface area respectively). For carbohydrates, starch and the reducing sugars of glucose, sucrose, fructose and maltose were tested for in the below-ground portions of field plants, and above-ground and below-ground portions of cultured plants. Starch was not detected in both above-ground and below-ground plant portions of both field and culture studies. Glucose content was highest among the four sugars, in both field and culture plants but not significantly different compared to the control. Changes in growth rates were the most discernible where increased shading results in decreased growth rates (3.72 $\pm$ 0.51 mm $apex^{-1}$ day^{-1} from control plants, to the significantly lowest recorded growth rate value of 0.746 $\pm$ 0.205 mm $apex^{-1}$ day^{-1}, $p < 0.01$, from Tank 1 plants). Leaf morphology based on leaf length, leaf width, leaf petiole length, number of cross veins per leaf, leaf fresh weight and leaf surface area were significantly higher for leaves under shading in culture condition compared to field-shaded leaves and the control. This is substantiated by the data from Tank 2 where leaf length is 24.73 $\pm$ 0.54 mm, leaf width – 9.38 $\pm$ 0.23, leaf length-width ratio – 2.80 $\pm$ 0.030, leaf petiole length – 28.48 $\pm$ 1.03, leaf cross vein number – 14.47 $\pm$ 0.27, leaf fresh weight – 0.0179 ± 0.00134 and leaf surface area – 2.011 ± 0.126) compared to the unshaded control (leaf length: 13.20 ± 0.54 mm; leaf width: 6.81 ± 0.29; leaf length-width ratio: 1.93 ± 0.037; leaf petiole length: 11.20 ± 1.43; leaf cross vein number: 11.40 ± 0.35; leaf fresh weight: 0.00680 ± 0.000548; and leaf surface area: 0.796 ± 0.0744). For field biomass values, there were no significant differences between shaded plants and the control. Comparatively, culture biomass values of Tank 1 were significantly higher for both above-ground biomass (0.0127 ± 0.00238 g DW $rhizome^{-1}$, $p < 0.01$) and below-ground biomass (0.0282 ± 0.00245 g DW $rhizome^{-1}$, $p < 0.01$) compared to the unshaded control (0.0107 ± 0.000914 g DW $rhizome^{-1}$ and 0.0192 ± 0.00109 g DW $rhizome^{-1}$ for above-ground and below-ground biomass respectively). All the observations and results collated showed *H. ovalis* tolerates extreme low light conditions as low as 96% shading (80 µmol m^{-2} s^{-1}) by modifying its various physical and biochemical characteristics accordingly with its light environment. This is also evident that the plant survives and continues to maintain productivity with respect to photosynthesis and carbohydrate production even under the highest shading levels imposed in both field (95% shading) and cultures (Tank 2 – 96% shading). Furthermore, it is possible to culture *H. ovalis*, although maximum growth densities equivalent to those observed in the field were not achieved. The findings suggest that lowered light availability may not be the sole causal factor for *H. ovalis* loss in a particular area. Other aspects such as epiphytic fouling and available nutrients could be more important in the loss of *H. ovalis* vegetation, although an interaction of the factor of reduced light and these other factors should not be discounted.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master of Science

SHADING RESPONSES OF THE SEAGRASS *HALOPHILA OVALIS* (R. BR.) HOOK. F.FROM TELUK KEMANG, NEGRI SEMBILAN, MALAYSIA

Oleh

MOHAMMAD ROZAIMI B JAMALUDIN

Jun 2008

Pengerusi: Japar Sidik Bujang, PhD

Fakulti: Sains

Kajian terhadap *Halophila ovalis* dari Teluk Kemang (2 ° 30'N, 101 ° 45'E), Port Dickson, Negeri Sembilan telah dibuat untuk melihat tindakbalas rumput laut ini kepada keredupan tiruan. Tindakbalas berdasarkan produktiviti autotrofik *H. ovalis* melalui beberapa eksperimen fotosintesis adalah untuk mengenalpasti kesan adaptasi tumbuhan kepada di lapangan (pertumbuhan semulajadi) atau di dalam kultur (cahaya dikurangkan ke 85-90% dari keamatan cahaya semulajadi). Hasil pemerhatian mendapati nilai kepampasan cahaya adalah tidak berbeza di antara daun dari lapangan atau daun dari kultur (8-13 µmol m^{-2} s^{-1}). Manakala titik ketepuan cahaya adalah berada dalam linkungan 268-275 µmol m^{-2} s^{-1} bagi daun dari lapangan dan nilai titik ketepuan cahaya bagi daun dari kultur meningkat ke linkungan 290-293 µmol m^{-2} s^{-1}. Kajian selama satu bulan telah dibuat terhadap tumbuhan di lapangan (tahap 50%, 65%, 80% dan 95% daripada intensiti cahaya lapangan) dan di dalam kultur (keredupan 92% pada Tangki 1 dan 96% keredupan pada Tangki 2) berbanding dengan kawalan tanpa keredupan

cahaya. Kadar fotosintesis *H. ovalis* di lapangan pada aras air surut dan pasang sederhana dan juga daripada kultur berdasarkan kaedah botol ‘Biological Oxygen Demand’ adalah sehingga enam kali lebih tinggi dari nilai yang didapati melalui kaedah elektrod oksigen. Kandungan klorofil pada daun tumbuhan di lapangan dan kultur yang diredupkan adalah lebih tinggi berbanding dengan kawalan di mana daun dari kultur (Tangki 2) menunjukkan nilai kandungan klorofil tertinggi (1353.40 $\pm$ 74.00 µg klorofil *a* g^{-1}, $p < 0.01$ bagi berat daun segar, dan 11.92 $\pm$ 0.59 µg klorofil *a* cm^{-2}, $p < 0.01$, bagi kawasan permukaan daun, serta 744.30 $\pm$ 46.55 klorofil *b* g^{-1} , $p < 0.01$ bagi berat daun segar dan 6.56 $\pm$ 0.39 µg klorofil *b* cm^{-2} , $p < 0.01$, bagi kawasan permukaan daun). Untuk kandungan karbohidrat, kanji dan empat jenis gula – glukos, sukros, fruktos dan maltos telah diuji pada bahagian tumbuhan yang di atas permukaan substrat (“above-ground”) dan di bawah substrat (“below-ground”) untuk di lapangan dan kultur. Kanji tidak dikesan pada kedua-dua bahagian tumbuhan “above-ground” dan “below-ground” untuk tumbuhan di lapangan dan kultur. Kandungan glukos adalah yang tertinggi berbanding gula yang lain tetapi nilainya tidak jauh berbeza dengan tumbuhan kawalan. Analisis kadar pertumbuhan telah menunjukkan nilai perbezaan yang paling ketara di mana didapati peningkatan kadar keredupan menyebabkan penurunan kadar pertumbuhan (pertumbuhan sebanyak 3.72 $\pm$ 0.51 mm $apex^{-1}$ $hari^{-1}$ bagi tumbuhan kawalan berbanding dengan tumbuhan pada Tangki 1 yang menunjukkan rekod nilai pertumbuhan yang paling rendah iaitu pada 0.746 $\pm$ 0.205 mm $apex^{-1}$ $hari^{-1}$, $p < 0.01$). Morfologi daun berdasarkan parameter kepanjangan daun, kelebaran daun, nisbah panjang-kelebaran daun, kepanjangan ‘petiole’ daun, jumlah 'cross veins' untuk sehelai daun, berat daun segar, dan luas permukaan daun di dalam keadaan keredupan di lapangan dan kultur menunjukkan nilai kesemua parameter-parameter ini adalah lebih tinggi berbanding tumbuhan kawalan. Ini disokong oleh data dari Tangki 2 di mana panjang daun adalah 24.73 ± 0.54 mm, kelebaran daun – 9.38 ± 0.23, nisbah panjang-kelebaran daun – 2.80 ± 0.030, kepanjangan 'petiole' daun – 28.48 ± 1.03, jumlah 'cross vein' daun – 14.47 ± 0.27, berat daun segar – 0.0179 ± 0.00134 dan kawasan permukaan daun – 2.011 ± 0.126 jika dibandingkan dengan tumbuhan kawalan (panjang daun: 13.20 ± 0.54 mm; kelebaran daun: 6.81 ± 0.29; nisbah panjang-kelebaran daun: 1.20 ± 1.43; kepanjangan 'petiole' daun: 11.40 ± 0.35; jumlah 'cross vein' daun: 14.47 ± 0.27; berat daun segar: 0.00680 ± 0.000548; dan kawasan permukaan daun: 0.796 ± 0.0744). Bagi nilai biojisim, tiada perbezaan ketara antara tumbuhan yang diredup di lapangan dan tumbuhan kawalan. Secara bandingan, nilai biojisim bagi tumbuhan dari Tangki 1 adalah lebih tinggi (0.0127 ± 0.00238 g DW rhizome^{-1}, $p < 0.01$, bagi bahagian di atas permukaan substrat dan 0.0282 ± 0.00245 g DW rhizome^{-1}, $p < 0.01$, bagi bahagian di bawah substrat) berbanding tumbuhan kawalan (0.0107 ± 0.000914 g DW rhizome^{-1} bagi bahagian di atas permukaan substrat dan 0.0192 ± 0.00109 g DW rhizome^{-1} bagi bahagian di bawah substrat). Berdasarkan kesemua pemerhatian dan hasil tinjauan yang telah dijalankan, didapati *H. ovalis* adalah toleran kepada keadaan keamatan cahaya yang rendah di mana tumbuhan ini melalui perubahan secara fizikal dan biokimia, mengikut kedapatan cahaya di persekitarannya. Ini juga terbukti bahawa tumbuhan ini mampu hidup dan mengekalkan produktiviti walaupun pada tahap keredupan yang tinggi, iaitu sebanyak 95% keredupan di lapangan dan sebanyak 96% keredupan di dalam kultur (Tangki 2). Adalah tidak mustahil untuk mengkulturkan *H. ovalis*, walaupun kadar maksimum bagi kepadatan pertumbuhan seperti tumbuhan di lapangan tidak tercapai. Hasil kajian ini memperlihatkan bahawa kerendahan terdapatan cahawa bukan hanya faktor yang menyebabkan kehilangan *H. ovalis* di sesuatu kawasan. Aspek-aspek lain seperti “epiphytic fouling” dan kedapatan nutrien berinteraksi dengan faktor kurangnya terdapatan cahaya perlu diambil kira juga.

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I certify than an Examination committee has met on the **12th of June, 2008** to conduct the final examination of **Mohammad Rozaimi b Jamaludin** on his **Master of Science** thesis entitled "**Shading responses of the seagrass *Halophila ovalis* (R. Br.) Hook. f. from Port Dickson, Negri Sembilan, Malaysia**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and the Universiti Pertanian Malaysia (Higher Degree) Regulations1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Aziz Arshad, PhD
Associate Professor
Faculty of Agriculture
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(Chairman)

Umi Kalsom Yusuf, PhD
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Phang Siew Moi, PhD
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HASANAH MOHD. GHAZALI, PhD
Pofessor and Deputy Dean
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Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Master of Science**. The members of the Supervisory committee were as follows:

Japar Sidik Bujang, PhD
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(Chairman)

Misri Kusnan, PhD
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Date: 14 August 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

MOHAMMAD ROZAIMI B JAMALUDIN

Date: 8th July 2008

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Photosynthetic efficiency
AG	Above-ground
Area	Leaf Surface Area
BG	Below-ground
BOD	Biological Oxygen Demand
Chl	Chlorophyll
DW	Leaf Dry Weight
FW	Leaf Fresh Weight
HPLC	High Performance Liquid Chromatography
I_c	Light compensation point
I_k	Light saturation point
IUCN	The World Conservation Union
KEGG	Kyoto Encyclopedia of Genes and Genomes
LHC	Light-Harvesting Complex
LHC II	Light Harvesting Complex II
NCSS	Number Cruncher Statistical System
PAR	Photosynthetically Active Radiation
P-I	Photosynthesis-Irradiance
P_{max}	Maximal photosynthetic capacity
PS I	Photosystem complex I
PS II	Photosystem complex II
R_{dark}	Dark respiration