RANDOMLY AMPLIFIED POLYMORPHIC DNA ANALYSIS OF SEAGRASS, *HALOPHILA OVALIS*

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INTRODUCTION

Seagrasses are an important component of coastal system, not only important as nursery ground and shelter for many species of marine life, but also have great value in stabilizing and protecting coastlines from erosion due to the physical character of their leaves and root-rhizome system (Sudara *et al.* 1992).

In recent years, as the importance of the communities of the coastal zone has been realized, the shallow seagrass beds have been studied with increasing intensity. Although there are several species of marine grasses in Malaysia, the main studies have centered on *Halophila ovalis* in Beting Tengah, Pulau Pinang and Pulau Besar, Johor (Bujang 1994).

RAPD (Random Amplified Polymorphic DNA) analysis employs single short primers with arbitrary sequences to generate genome-specific fingerprints of multiple amplification products (Welsh & McClelland 1990; Williams *et al.* 1990). RAPD markers have proved useful in many plant genetic studies. These include the determination of DNA diversity between *Saccharum* varieties (Harvey & Botha 1996) and identification and classification of *Pisum sativum* genotypes (Samec & Nasinec 1996). The objectives of the present work is to identify polymorphic RAPD markers useful for distinguishing among *H. ovalis* which are present in different locations.

MATERIAL AND METHODS

Plant materials

The source of material for the present study was obtained from two states in Peninsular Malaysia, namely Pulau Pinang and Johor. 71 samples of *H. ovalis* were collected from three locations in Beting Tengah (middle bank and 2 small islands near middle bank i.e. island 1 = 500 m south of middle bank and island 2 = 650 m south of middle bank)), Pulau Pinang and the others three locations in Pulau Besar (north west, west and south part of Pulau Besar), Johor. The samples were thoroughly cleaned with tap water, drying over silica gel and stored at -20°C until needed for DNA extraction.

DNA extraction

DNA was isolated from dried material using the hexadecyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980), with some modifications. The DNA was quantitated spectrophotometrically (OD_{260}=50 ng/µl) and stored at -20°C.

RAPD Analysis

Ten decamer oligonucleotides (Operon Technologies Inc.) were used for PCR amplification. Initially 40 primers were screened using the DNA from Pulau Pinang. Primers that exhibited good result were ultimately selected for further evaluation.

The amplification reactions with a total volume of 25 µl was used which contained 2.5 µl of Thermophilic DNA Polymerase 10 X buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTP, 1 pmol primer, 0.5 U *Taq* DNA Polymerase (Promega Coorporation, supplied in storage buffer B) and genomic DNA.The amplifications were carried out in an MJ Research thermal cycler (PTC-200) for an initial 1.5 min denaturation at 94°C followed by 35 cycles of 45 s at 94°C, 30 s at 40°C, 40 s at 50°C, 30 s at 60°C, 2 min at 72°C and a final stage of 6 min at 72°C. All PCR products were separated by electrophoresis on 1.5% w/v agarose gels in 0.5 X TBE buffer (Sambrook *et al.* 1989), stained with ethidium bromide and viewed under ultraviolet light.

Data scoring and analysis

The RAPD bands were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence of a band. These RAPD data were used to compile a binary matrix for cluster analysis using the RAPDistance program Version 1.03 (Armstrong *et al.* 1995). Genetic similarity among individuals was calculated according to Jaccard's similarity coefficients. Jaccard's coefficients are defined as a/(a+b+c), where a is the number of positive matches (i.e. bands common to two accessions), and b and c refer to the number of bands present only in two individuals i.e. 1 and 2 respectively. The similarity coefficients (Table 1) were then used to construct a dendrogram using Neighbor-Joining using the computer program PHYLIP (Felsenstein 1993). Finally, a dendrogram can be view under a TreeView program.

RESULTS AND DISCUSSION

Quality and Quantity of DNA

The quality and quantity DNA obtained range from 1.6 to 2.0 and 100 ng/µl to 500 ng/µl. In general, fresh leaves are used as the source of DNA for RAPD analysis. However, fresh laminas are not convenient sources of DNA when large-scale field collection is to be performed.

DNA amplification banding profile

Of the 40 primers [OPF-01 to OPF-20 and OPC-01 to OPC-20] tested, six primers [OPC-06: 5'-GAACGGACTC-3', OPC-07: 5'-GTTCCGACGA-3', OPC-08: 5'-TGGACCGGTG-3', OPC-19: 5'-GTTGCCAGCC-3', OPC-20: 5'-ACTTCGCCAC-3', OPF-05: 5'-CCGAATTCCC-3'] were ultimately selected for further evaluation that produced a total of 70 bands. The total number of bands produced per primer varied from 8 to 15 and the size of bands ranged from 300 to 1877 base pairs (bp). Representative RAPD profiles generated with OPC-19 for the *H. ovalis* studied are shown in Figure 1.

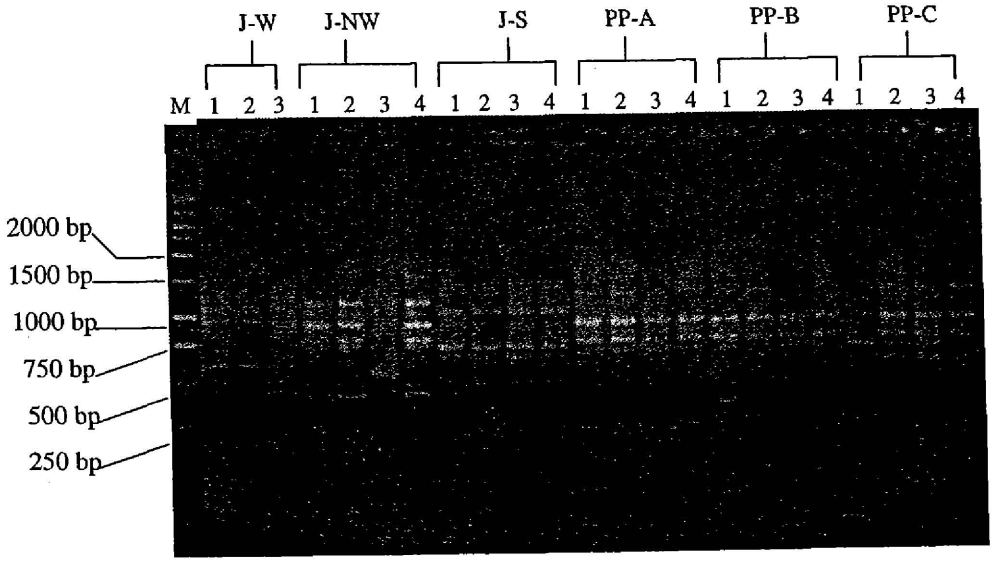

Figure 1: RAPD profiles generated with primer OPC-19 and separated by agarose (1.5% w/v) gel electrophoresis. Lane M. 1 Kbp DNA Ladder. J are samples from Johor and PP are the samples from Pulau Pinang.

Genetic distance analysis

Cluster analysis (neighbor-joining method) was used to generate a dendrogram (Figure 2). The 71 samples are separated into two main clusters i.e. individuals from Johor grouped into cluster I and individuals from Pulau Pinang are grouped in cluster II. We did not observe intralocation variations in the RAPD profiles in our study. This indicates that RAPD markers provide a more reliable method than morphological characters to identify closely related *H. ovalis*.

Table 1: Mean values of genetic similarity between major locations of *H. ovalis*

Locations	J-W	J-NW	J-S	PP-A	PP-B	PP-C
J-W, west of Pulau Besar	-					
J-NW, north west of Pulau Besar	0.298	-				
J-S, south of Pulau Besar	0.295	0.250	-			
PP-A, middle bank of Pulau Pinang	0.217	0.269	0.216	-		
PP-B, island 1, 500 m south of middle bank	0.217	0.269	0.216	-	-	
PP-C, island 2, 650 m south of middle bank	0.233	0.264	0.235	0.975	0.975	-

From three locations in Pulau Besar that we have study, it showed a very low mean genetic similarity, which was approximately 30% between each of them. While, the three

locations in Beting Tengah show a very high mean genetic similarity, which was 98% between each of.them.

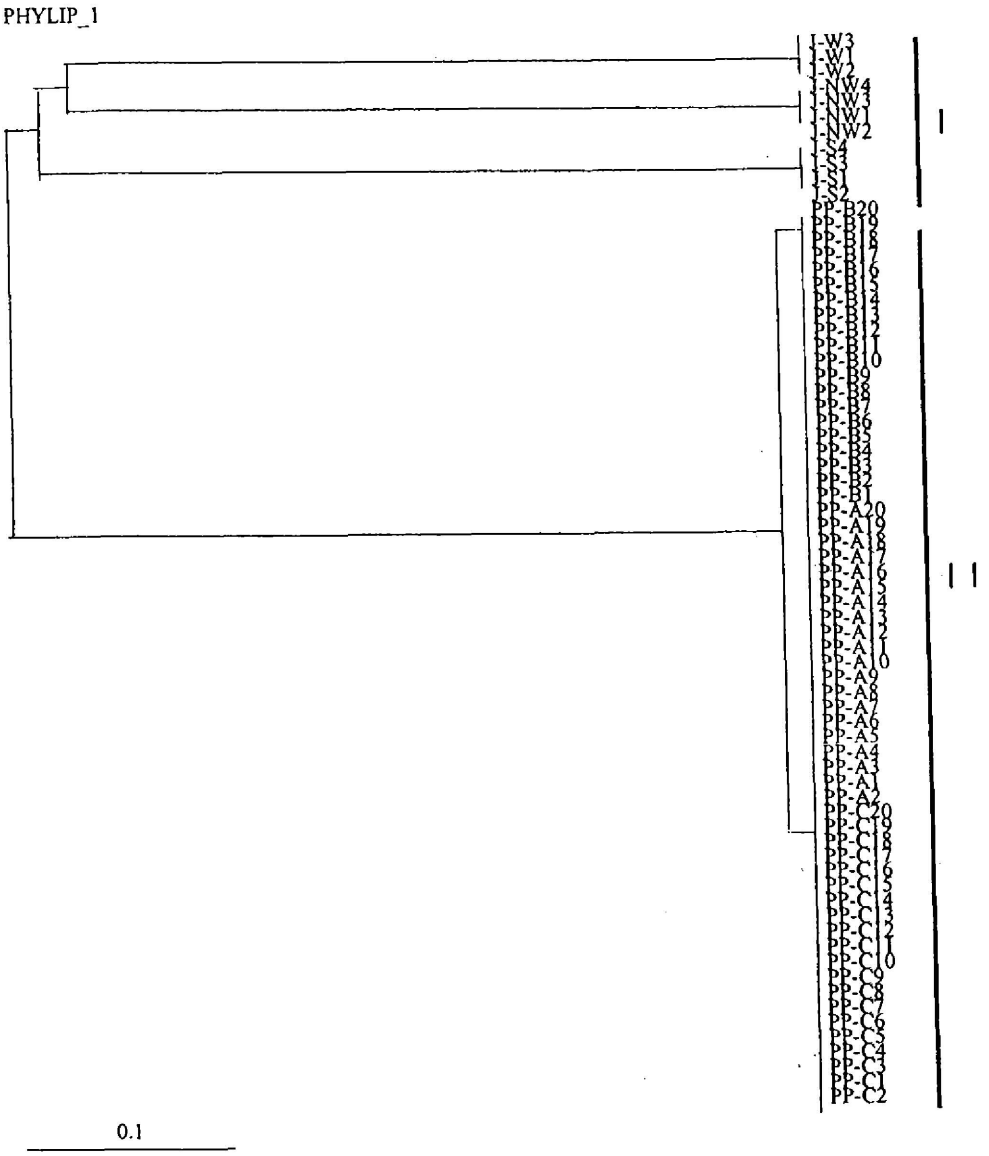

Figure 2: Neighbor-Joining cluster analysis of RAPD data generated by six random primers for *H. ovalis*.

If we compared the mean genetic similarity among locations in Johor and Pulau Pinang, it also shows a low value of genetic similarity, which ranged from 22% to 26%. *H. ovalis*

use both vegetative and sexual as a mode of reproduction. Although, populations are reproducing predominantly by vegetative means, a limited amount of sexual reproduction could account for the genetic variation. From this study, we predict that three locations of *H. ovalis* in Pulau Besar were isolated from each other and might originated from three different clones. However, those *H. ovalis* from Beting Tengah the influence of high and low tide couple with the perhaps a predominantly asexual reproduction which mean all the individuals are well distributed showing a high genetic similarity among them.

The present present study is a first step in elucidating the genetic variation in *H. ovalis* using RAPD markers. Further studies can be carried out to investigate more locations of *H. ovalis* in Malay Peninsular. Then, it may provide a better understanding of genetic variation in this species. We conclude that used correctly, RAPD analysis is a rapid, simple and reliable tool in estimating the genetic variation in *H. ovalis*.

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REFERENCES

Armstrong JAG, R Peakall and G Weiller 1995. *RAPDistance Program Version 1.03 for the Analysis of Pattern of RAPD Fragments*. Australian National University, Canberra.

Bujang J S 1994. Status of Seagrass Resources in Malaysia. *ASEAN – Australia Third Symposium on Living Coastal Resources, Chulalongkon University, Bangkok, Thailand, 16 – 20 May 1994.*

Felsenstein J 1993. *PHYLIP (Phylogeny Inferrence Package) Version 3.5s*. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Harvey M and F C Botha 1996. Use of PCR-based methodologies for the determination of DNA diversity between *Saccharum* varieties. *Euphytica*, 89, 257-265.

Murray M G and W F Thompson WF 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8, 4321-4325.

Sambrook J, E F Fritsh and T Maniatis 1989. *Molecular cloning: a Laboratory Manual.* Cold Spring Harbor Laboratory Press, New York.

Samec P and V Nainec 1996. The use of RAPD techniques for the identification and classification of *Pisum sativum* L. genotypes. *Euphytica*, 89, 229-234.

Sudara S, S Nateekarnjanalarp and S Plathong 1992. Survival and growth rate of transplanted seagrasses *Halodule pinifolia* and *Enhalus acoroides* in different types of substrata. *Third ASEAN Sciences and Technology Week Conference Proceedings*, Vol.6, 261-266.

Welsh J, M McClelland 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, 18, 7213-7218.

Williams J G K, A R Kubelik, K J Livak, J A Rafalski and S V Tingey 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research Acids*, 18, 6531-6535.