

STUDY ON GEOMETRIC MACHINABILITY OF ENGINEERING METALS
(Geometric Machinability) USING RPPD -
RDP TECHNIQUE

DIPAL SINGH DULAWALA

DEPARTMENT OF MECHANICAL ENGINEERING
NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE, MALAYSIA
2005

Perdustakaan
Kolej Universiti Sains Dan Teknologi Malaysia (KUSTEM)

1100036830

LP 37 FST 1 2005



1100036830

Study on genetic variability of flowery venus (*clausinella chlorotica*) using RAPD-PCR technique / Rizal Afenddy Anuar.



PERPUSTAKAAN

KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

1100036830

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

STUDY ON GENETIC VARIABILITY OF FLOWERY VENUS (*Clausinella chlorotica*) USING RAPD – PCR TECHNIQUE

By
Rizal Afenddy bin Anuar

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

This project should be cited as:

Rizal-Afenddy, A. 2004. Study on genetic variability of Flowery Venus (*Clausinella chlorotica*) using RAPD – PCR technique. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 74p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor it be stored in a retrieval system, transmitted or otherwise copied for public or private use without written permission from the author and the supervisor(s) of the project.



**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: Study on genetic variability of Flowery Venus (*Clausinella chlorotica*) using RAPD - PCR technique oleh Rizal Afenddy Bin Anuar No. Matrik UK6457, telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains-Sains Biologi Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

.....
Penyelia Utama

WAN BAYANI WAN OMAR

Nama:

PENSYARAH

Cop Rasmi:

**Jabatan Sains Biologi
Fakulti Sains & Teknologi**

Tarikh: *7/4/2005*

**Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu, Terengganu**

.....
Penyelia Kedua (jika ada)

Nama:

*Dr. Zaleha Binti Kassim,
Pensyarah*

Cop Rasmi

*Jabatan Sains Samudera
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.*

Tarikh:

7/4/05

.....
Ketua Jabatan Sains Biologi

Nama:

*PROF. MADYA DR. NAKISAH BT. MAT AMIN
Ketua*

Cop Rasmi:

*Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.*

Tarikh:

7/4/05

ACKNOWLEDGEMENTS

First of all, I would like to thank to Allah S.W.T for giving me the strength to finish this thesis and the blessing that I needs to accomplish this work. I would like to express my sincere appreciation to my supervisor, Cik Wan Bayani Wan Omar and also my co-supervisor Dr. Zaleha Kassim from Department of Biological Science and Samudera Science, Faculty of Science and Technology for their guidance, advice, encouragement and understanding. Without their cooperation, patience and full support, this project will not survive. I also wish to convey my appreciation to my family for their moral support and understanding.

I am grateful to the Faculty of Science and Technology, KUSTEM for allowing me to use the facilities and also to the staff, En. Mazrul in helping me to use the laboratory apparatus.

My heartiest gratitude and big thank you goes especially to my project partners, who always willing to give their fully support during the process of completing this project. Thanks for your encouragement, caring, understanding and patience.

Lastly, I would like to dedicate my appreciation to all my friends, my family and course mates who had comfort me during my happy and sad time in this project. Thanks for your concern very much. Thank you.

TABLE OF CONTENTS

Title	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF APPENDICES	ix
LIST OF ABBREVIATIONS	x
ABSTRACT	xii
ABSTRAK	xiii
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	
2.1 Taxonomy and Morphology	4
2.2 Habitat and Distribution	6
2.3 Feeding and Reproduction	6
2.4 Genetic Variation	8
2.5 DNA Polymorphism	9
2.6 Molecular Genetic Marker	9
2.7 Polymerase Chain Reaction	10

2.8	Random Amplified Polymorphic DNA	13
2.9	Gel Electrophoresis of DNA	14

CHAPTER 3 MATERIALS AND METHODS

3.1	Collection of sample, <i>Clausinella chlorotica</i>	16
3.2	DNA Extraction	
	3.2.1 Wizard Genomic DNA Purification Kit (Promega)	19
3.3	Agarose Gel Electrophoresis	20
3.4	Measurement of DNA Purity and Quantity	20
3.5	Screening of RAPD Primers	21
3.6	DNA Amplification of Selected RAPD Primers	22
3.7	Data Analysis	23

CHAPTER 4 RESULT

4.1	Purity and Quantity of DNA	25
4.2	Screening of RAPD Primers	28
4.3	RAPD Profiles	28
4.4	Dendrogram Analysis	
	4.4.1 Genetic Distance	34
	4.4.2 Similarity Index	35

CHAPTER 5 DISCUSSION

5.1	Purity and Quantity of DNA	42
5.2	Screening of RAPD Primers	45
5.3	RAPD Profiles	46

5.4	Dendrogram Analysis	49
CHAPTER 6 CONCLUSION AND RECOMMENDATION		52
REFERENCES		54
APPENDICES		59
CURRICULUM VITAE		73

LIST OF TABLES

Table		Page
3.1	Code, sequence, nucleotide length and G+C content of primers used in Random Amplified Polymorphic DNA analysis.	21
4.1	DNA Purity and Quantity of <i>C. chlorotica</i> at Pulau Che Him.	26
4.2	DNA Purity and Quantity of <i>C. chlorotica</i> at Pulau Semut.	26
4.3	Fragment number and length of <i>C. chlorotica</i> DNA after amplified using various primers.	34
4.4	Total number of fragments, polymorphic fragments, proportion of polymorphism and size range of fragments of RAPD of <i>C. chlorotica</i> from two different location in Setiu Wetland, Terengganu.	34
4.4	Similarity index of <i>C. chlorotica</i> from Pulau Che Him.	41
4.5	Similarity index of <i>C. chlorotica</i> from Pulau Semut.	41

LIST OF FIGURES

Figure	Page
2.1 The classification of <i>Clausinella chlorotica</i> .	5
3.1 Sample of <i>Clausinella chlorotica</i> used in this study.	17
3.2 Picture of location of the sampling area at Setiu Wetland, Terengganu.	18
4.1 Genomic DNA extracted by Wizard Genomic DNA Purification Kit (Promega) protocol from Pulau Che Him.	27
4.2 Genomic DNA extracted by Wizard Genomic DNA Purification Kit (Promega) protocol Pulau Semut.	27
4.3 RAPD banding patterns for screening of Operon Technology Kit A primers, OPA 01 to OPA 10.	30
4.4 RAPD banding patterns for screening of Operon Technology Kit A primers, OPA 11 to OPA 20.	30
4.5 Banding pattern of RAPD fragments of <i>C. chlorotica</i> from Pulau Che Him using primer OPA 02.	31
4.6 Banding pattern of RAPD fragments of <i>C. chlorotica</i> from Pulau Che Him using primer OPA 03.	31
4.7 Banding pattern of RAPD fragments of <i>C. chlorotica</i> from Pulau Che Him using primer OPA 18.	32
4.8 Banding pattern of RAPD fragments of <i>C. chlorotica</i> from Pulau Semut using primer OPA 02.	32
4.9 Banding pattern of RAPD fragments of <i>C. chlorotica</i> from Pulau Semut using primer OPA 03.	33

4.10	Banding pattern of RAPD fragments of <i>C. chlorotica</i> from Pulau Semut using primer OPA 18.	33
4.11	Dendrogram showing genetic relationship between individuals of the <i>C. chlorotica</i> population collected in Pulau Che Him.	37
4.12	Dendrogram showing genetic relationship between individuals of the <i>C. chlorotica</i> population collected in Pulau Semut.	38
4.13	Dendrogram showing genetic relationship between 12 individuals of the <i>C. chlorotica</i> population collected in Pulau Che Him and Pulau Semut.	39
4.14	UPGMA cluster analysis based on the genetic distance (dissimilarity index) generated from Nei and Li's indices of different <i>C. chlorotica</i> population.	40

LIST OF APPENDICES

Appendix	Page
A Length, width and body weight of <i>Clausinella chlorotica</i> from different population.	60
B1 Bands score of OPA 02 from Pulau Che Him.	61
B2 Bands score of OPA 03 from Pulau Che Him.	62
B3 Bands score of OPA 18 from Pulau Che Him.	63
B4 Bands score of OPA 02 from Pulau Semut.	64
B5 Bands score of OPA 03 from Pulau Semut.	65
B6 Bands score of OPA 18 from Pulau Semut.	66
C1 Dissimilarity index Pulau Che Him.	67
C2 Dissimilarity index Pulau Semut.	67
C3 Dissimilarity index for both population Pulau Che Him and Pulau Semut.	68
C4 Similarity index for both population Pulau Che Him and Pulau Semut.	69
D Apparatus needed for this study.	70

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
1X	One Time
A	Adenosine
bp	Base pair
C	Cytosine
cm	Centimeter
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
dNTP mix	Deoxyribonucleotides mixture
EDTA	Ethylenediaminetetraacetic acid
g	Gram
G	Guanocine
M	Molarity
µg	Microgram
µL	Microlitre
µM	Micromolar
mg	Miligram
mL	Mililitre
mM	Milimolar

min	Minutes
ng	Nanogram
OD	Optical density
PCR	Polymerase Chain Reaction
Pmole	Picomole
Ppt	Part per trillion
RAPD	Random Amplified Polymorphic DNA
rpm	Rotation per minute
sec	Seconds
SD	Standard Deviation
T	Thymine
TBE	Tris-borate-EDTA buffer
TE	10mM Tris Cl, 1 mM EDTA
Tris-HCL	Tris [Hydroxymethyl] aminomethane hydrochloride
UV	Ultra violet
V	Volt
VDS	Video Documentation System
v/v	volume/volume
w/v	weight/volume

ABSTRACT

The main objectives of this study are to assess the degree of polymorphism of *Clausinella chlorotica* by using RAPD-PCR technique and to establish the genetic database on the genetic variability of *C. chlorotica*. The Random Amplified Polymorphic DNA (RAPD) in association with Polymerase Chain Reaction (PCR) was used to examine the genetic variability and relationship among individuals within and between populations of *C. chlorotica* from Pulau Che Him and Pulau Semut, Setiu Wetland, Terengganu. The genomic DNA was extracted from the clam tissues by using Kit WizardTM Genomic DNA Purification (Promega). Twenty oligonucleotide primers were screened and only three primers were selected, OPA 02, OPA 03, and OPA 18 to amplify DNA from twelve samples of *C. chlorotica* from the two populations. A total of 55 RAPD fragments with size ranging between 250- 1750 bp were generated and 39 of them were polymorphic. Genetic distance level between two populations range from 0.05 to 0.7692 and the polymorphism detection in sample from Pulau Che Him was 71.88% and from Pulau Semut was 69.57%. The total of polymorphism fragment from both locations was 70.91%.

KAJIAN MENGENAI KEPELBAGAIAN GENETIK *Clausinella Chlorotica* DENGAN MENGGUNAKAN TEKNIK RAPD – PCR

ABSTRAK

Tujuan penyelidikan ini ialah untuk menentukan darjah polimorfisme bagi siput *Clausinella chlorotica* dengan menggunakan teknik RAPD-PCR dan juga untuk menubuhkan pangkalan data kepelbagaian genetik bagi siput ini. Dalam kajian ini, polimorfisme DNA rawak teramplifikasi (RAPD) bersama dengan tindak balas berantai polimorfisme (PCR) telah digunakan bagi menentukan kepelbagaian dan pertalian genetik di antara individu-individu di dalam dan di antara populasi-populasi siput *C. chlorotica* dari Pulau Che Him and Pulau Semut, Setiu Wetland, Terengganu. Pengekstrakan DNA daripada tisu siput dijalankan dengan menggunakan kaedah ‘WizardTM Genomic DNA Purification Kit’. Dua puluh pencetus telah diuji dan hanya tiga pencetus iaitu OPA 02, OPA 03 dan OPA 18 telah dipilih untuk mengamplifikasi DNA daripada 12 sampel yang mewakili dua populasi tersebut. Sejumlah 55 jalur segmen RAPD dengan saiz antara 250-1750 bp telah dihasilkan dan 39 daripadanya adalah polimorfik. Jarak perbeaan genetik di antara populasi adakah dari 0.05 ke 0.7692. Peratus jalur polimorfik yang didapati dari sampel bagi Pulau Che Him adalah 71.88% dan dari Pulau Semut adalah 69.57%. Jumlah keseluruhan bagi jalur polimorfik untuk kedua-dua lokasi adalah 70.91%.