

SCREENING OF DIFFERENT ALUMINOSILATE RESISTANCE
AND SUSCEPTIBLE BIOTYPES OF *Blattella*
indica (L.) Gerts. WITH RAPDs

TEH SABARINAH BINTI ABD MANAN

FAKULTAS SAINS DAN TEKNOLOGI
UNIVERSITI MALAYA KUALA LUMPUR SEREMBANG
2007

1100051186



LP 73 FST 2 2007



1100051186

Screening of different glyphosate resistance and susceptible biotypes of *Eleusine indica* (L.) Gaertn with Rapds / Teh Sabariah Abd Manan.

PERPUSTAKAAN
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

	100051186	

Lihat sebelah

HAK MILIK
PERPUSTAKAAN UMT

SCREENING OF DIFFERENT BIOTYPES OF GLYPHOSATE RESISTANCE
AND SUSCEPTIBLE BIOTYPES *Eleusine indica* (L) Gaertn WITH RAPDs

By

Teh Sabariah Binti Abd Manan

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
UNIVERSITI MALAYSIA TERENGGANU
2007

1100051186

This project should be cited as:

Teh Sabariah, A. M. 2007. Screening of Different Biotypes of *Eleusine indica* (L) Gaertn With RAPDs. Undergraduate thesis, Bachelor of Biological Science, Faculty of Science and Technology, University Malaysia Terengganu. 55p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may 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
UNIVERSITI MALAYSIA TERENGGANU

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II
RESEARCH REPORT VERIFICATION**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **SCREENING OF DIFFERENT GLYPHOSATE RESISTANCE AND SUSCEPTIBLE BIOTYPES OF *Eleusine indica* WITH RAPDs** oleh **TEHSABARIAH BINTI ABD. MANAN**, no. matrik: **UK10504** telah diperiksa dan semua pembedahan 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, Universiti Malaysia Terengganu.

Disahkan oleh: / Verified by:


.....
Penyelia Utama / Main Supervisor

Nama: DR CHA THYE SAN

Cop Rasmi:

DR. CHA THYE SAN
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: 9/5/2007


.....
Penyelia Kedua / Co-Supervisor

Nama: DR CHUAH TSE SEN

Cop Rasmi:

DR. CHUAH TSE SEN
Pensyarah
Jabatan Agroteknologi
Fakulti Agroteknologi dan Sains Mula
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: 15/5/2007


.....
Ketua Jabatan Sains Biologi / Head, Department of Biological Sciences

Nama: DR. AZIZ B. AHMAD

Cop Rasmi:

DR. AZIZ BIN AHMAD
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 10/5/2007

ACKNOWLEDGEMENT

Assalamualaikum wam and greetings to readers,

Alhamdulillah, I feel grateful to Allah for His blessings and guidance upon my studies and living life as a student in University Malaysia Terengganu. Course changed is one important decision have been made in my life. Undeniable, it is not an easy way to get through but finally done in 5 semester of dazzling buzz. Supportive friends of juniors and peers from Bioscience, Biodiversity, Chemistry and Computational Mathematics program helps me a lot. Thanks guys. My housemates; Aisyah, Kak Gee, Kak Wa, Nelly, Betty. We treasure our best moments together in 44, Taman Desa Sri Tanjung.

This study is a kick-start for me to widen my horizon in learning. My paces have been guided by lecturers. A heartiest appreciation to Dr Cha Thye San. He never failed in giving us his commitment to the fullest. We are the luckiest students to have you as our supervisor. Hoping for the dreams to build a biotech industry plant soon will become reality. A warmth gratitude to Dr Chuah Tse Seng for his supports. Supplying all the goosegrass seeds, giving his guide in growing the rumets of goosegrass and not to forget, for his guide in analytical statistics for my other subjects. He's a good person that you can rely on when it comes to statistic. Thank you.

My supportive, friendly lab friends and also my course-mates supervised by Dr Cha; Tan, Banreet, Adlene, Hoi Fen, Steven, Liang, Hong Yew, Fong, my best buddy Betty and Jack, my research partner. Keeping track in all the nicest, sweetest and cheekiest memories together. I will miss you guys.

My parents who always have the time for me and understanding siblings of mine; Along, Merul and Aira and my fiance, Ahmad Shafie who always be there when I need them most. Thank you very much.

CONTENTS

	PAGE
ACKNOWLEDGEMENT	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
LIST OF ABBREVIATIONS	vi
LIST OF APPENDICES	vii
ABSTRACT	viii
ABSTRAK	ix
CHAPTER 1	INTRODUCTION
1.1	Introduction 1
1.2	Importance of Study 3
1.3	Objectives 3
CHAPTER 2	LITERATURE REVIEW
2.1	<i>Eleusine indica</i> (L.) Gaertn 4
2.1.1	Morphological Appearance and Thriven 4
2.1.2	Molecular Characteristic and Mechanism of Mutation 5
2.1.3	Research on Glyphosate Resistant Biotypes of Goosegrass 6
2.1.4	Unforeseen Consignment on Weed Management 7
2.2	Random Amplified Polymorphic DNA 9
CHAPTER 3	METHODOLOGY
3.1	Seeds Reservoir 11

3.1.1	Seed Processing and Sowing	11
3.1.2	Screening the Biotype of <i>Eleusine indica</i>	11
3.2	Genomic DNA Extraction from <i>Eleusine indica</i>	12
3.3	Gradient Polymerase Chain Reaction	13
3.4	Screening of Different Biotypes of <i>Eleusine indica</i> with Selectable Markers	14
CHAPTER 4	RESULTS	
4.1	Greenhouse Experiment	16
4.2	Gradient PCR	18
4.3	RAPD PCR	23
CHAPTER 5	DISCUSSION	28
CHAPTER 6	CONCLUSION AND RECOMMENDATIONS	
6.1	Conclusion	32
6.2	Recommendations	33
REFERENCES		34
APPENDICES		37
CURRICULUM VITAE		38

LIST OF TABLES

Table		Page
Table 3.1	DNA quantification of goosegrass from five different population.	15
Table 3.2	Sequences of 10-base nucleotides primers used for screening biotypes of goosegrass with RAPD	17
Table 4.1	Different populations of goosegrass with respective whole plants assay through a wide range sprayed of herbicide doses resulting in five different biotypes.	20
Table 4.2	The optimal PCR conditions for DNA annealing temperature for RAPD primers.	28
Table 4.3	RAPD markers for susceptible and resistant biotypes of <i>Eleusine indica</i> .	35

LIST OF FIGURES

Figure		Page
4.1	Genomic DNA profile for every biotypes of <i>Eleusine indica</i> .	21
4.2	Gradient PCR profiles for RAPD primers OPA-05 and OPA-06.	23
4.3	Gradient PCR profiles for RAPD primers OPA-12 and OPA-14.	24
4.4	Gradient PCR profiles for RAPD primers OPA-15 and OPA-20.	26
4.5	RAPD profiles for RAPD primers OPA-05 and OPA-06.	30
4.4	RAPD profiles for RAPD primers OPA-12 and OPA-14.	31
4.5	RAPD profiles for RAPD primers OPA-15 and OPA-20.	33

LIST OF ABBREVIATIONS

AFLP	-	Arbitrarily Fragment Length Polymorphism
AP-PCR	-	Arbitrarily Primed Polymerase Chain Reaction
DAF	-	DNA Amplification Fingerprinting Technique
EPSPS	-	5-enolpyruvylshikimate synthase
OD ₂₆₀	-	Absorbance value at 260 wavelengths
OD ₂₈₀	-	Absorbance value at 280 wavelengths
ng.μ L ⁻¹	-	Nanogram per microlitre
μ.L	-	Microlitre
g.a.i.ha ⁻¹	-	active ingredient per hectar
kg.ae.ha-1	-	active equivalent per hectar
PCR	-	Polymerase Chain Reaction
PEP	-	Phosphoenol pyruvate
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
SCARs	-	Sequence Characterized Amplified Regions Analysis
S3P	-	Shikimate-3-phosphate

LIST OF APPENDICES

Appendices	Page
Appendix 1 The populations of <i>Eleusine indica</i> grown in University Malaysia Terengganu greenhouse.	54

ABSTRACT

Goosegrass (*Eleusine indica*) is a monocot weed in the Poaceae family and distributes in many areas through out the world. This study was carried out to screen the different biotypes of *Eleusine indica* with RAPD and to identify RAPD markers for each resistance levels of goosegrass. Five populations of *Eleusine indica* from various places of plantation area in Kedah and Perak were screened with glyphosate-sprayed at different level of doses along with a standardized condition in University Malaysia Terengganu greenhouse for two weeks. The results revealed that five different biotypes; consist of susceptible, 2-folds, 4-folds, 8-folds and the highest resistant level observed was 11-folds. These populations underwent RAPD screening of six primers; OPA-05, OPA-06, OPA-12, OPA-14, OPA-15 and OPA-20 resulting in 8 different random amplified polymorphic DNA markers. This study clarified the use of polymorphism exhibited by random amplified polymorphic DNA of *Eleusine indica* in generating a particular genetic marker for each biotypes and totally beneficial for weed management problem in the future.

**PENYARINGAN BIOTIP RINTANG DAN SENSITIF YANG BERBEZA
PADA RUMPUT SAMBAU (*Eleusine indica*) TERHADAP GLIFOSAT
MENGUNAKAN KAEDAH RAPD**

ABSTRAK

Rumput sambau (*Eleusine indica*) merupakan rumput monokot daripada famili Poaceae dan mudah didapati hampir di seluruh dunia. Kajian ini bertujuan untuk membuat saringan perbezaan biotip (biotype) bagi *Eleusine indica* dengan teknik RAPD dan untuk mengenalpasti penanda RAPD bagi setiap tahap kerintangan pada rumput sambau. Proses penyaringan dibuat pada lima populasi rumput sambau yang diperolehi dari kawasan pertanian yang berlainan di Perak dan Kedah menggunakan kaedah penyemburan herbisid dari jenis glifosat. Semua populasi ini dibiak dan dibesarkan dengan penjagaan rapi di rumah hijau Universiti Malaysia Terengganu. Penyemburan herbisid dilakukan selepas rumput sambau berusia dua minggu. Primer-primer RAPD yang digunakan ialah OPA-05, OPA-06, OPA-12, OPA-14, OPA-15 dan OPA-20. Hasil ujian saringan di rumah hijau mendapati kelima-lima populasi mempunyai 5 jenis tahap kerintangan berbeza iaitu sensitif (tiada kerintangan), 2-lipatan, 4-lipatan, 8-lipatan dan 11-lipatan tahap kerintangan. Kesemua populasi ini melalui saringan RAPD. Hasilnya, 8 penanda RAPD diperolehi. Sekaligus membuktikan kelebihan polimorfisme melalui RAPD boleh menjana penanda RAPD untuk mengesan tahap kerintangan berbeza bagi rumput sambau.