UNIVERSITI PUTRA MALAYSIA

GENETIC VARIATION IN MALAYSIAN *Cymbopogon citratus* (LEMONGRASS) BASED ON INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKER

WAN NURFATIN WAN MOHD JAAFAR

FBSB 2015 172

GENETIC VARIATION IN MALAYSIAN *Cymbopogon citratus* (LEMONGRASS) BASED ON INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

WAN NURFATIN BINTI WAN MOHD JAAFAR

BACHELOR OF SCIENCE (HONS.)
UNIVERSITI PUTRA MALAYSIA
2015

TABLE OF CONTENTS	PAGE
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENT	iv
APPROVAL	v
DECLARATION	vi
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	x
CHAPTERS	
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	
2.1 Introduction to *Cymbopogon citratus*	4
2.2 The Importance of *Cymbopogon citratus*	5
2.3 Genetic variation in plants	8
2.4 Inter Simple Sequence Repeat	9
3.0 MATERIALS AND METHOD	
3.1 Sample collection	12
3.2 DNA extraction	15
3.3 DNA Quantification	16
3.4 ISSR markers selection	16
3.5 Scoring and Data Analysis	18
4.0 RESULTS AND DISCUSSION	
4.1 Diversity assessment of *C. citratus* phenotype	20
4.2 ISSR markers evaluation	22
4.3 Genetic variation within species	26
4.4 Phylogenetic relationship	27
5.0 CONCLUSION AND RECOMMENDATIONS	29
6.0 REFERENCES	30
7.0 APPENDICES	34

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfilment of the requirement for the degree of Science

Genetic Variation in Malaysian *Cymbopogon citratus* (Lemongrass) Based on Inter Simple sequence Repeat (ISSR) Markers

By

Wan Nurfatin binti Wan Mohd Jaafar

June 2015

Chair : Dr. Noor Baity Binti Saidi

Faculty: Faculty of Biotechnology and Biomolecular Sciences

This research was to investigate the genetic variation among *Cymbopogon citratus* sp. or commonly known as lemongrass in Malaysia. A total of 39 *C. citratus* were obtained from across Malaysia. Using the CTAB extraction method, DNA was extracted from the 39 samples and inter simple sequence repeat (ISSR) markers were used to estimate their genetic variation. Out of 25 ISSR primers tested, only seven ISSR primers were chosen based on their ability to amplify DNA fragments that were easy to score on an agarose gel and reproducible. In total 70 loci were identified and 59 were found to be polymorphic with an average of 8.43 polymorphic loci per primer. Using the POPGENE program values of Shannon index was calculated to estimate the genetic diversity of *C. citratus* and the highest values obtained is 0.2263 which indicate low level of genetic diversity. Genetic distances were also calculated using the GenAlEx program and the average for the genetic distance of all samples is 16.35. From the data obtained, a phylogenetic tree was constructed using the UPGMA method. Three cluster were generated, where 2 cluster share the same branch, and the third cluster in the second branch.

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan ijazah Sains

Variasi Gen dalam *Cymbopogon citratus* (Serai makan) Malaysia berdasarkan Penanda Inter Simple Sequence Repeat (ISSR)

Oleh

Wan Nurfatin Binti Wan Mohd Jaafar

Jun 2015

Pengerusi: Dr. Noor Baity Binti Saidi, PhD

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Kajian ini diadakan untuk menyiasat variasi dalam gen *Cymbopogon citratus* spesis atau lebih dikenali sebagai serai makan di Malaysia. Secara keseluruhannya, sebanyak 39 sampel *C.citratus* diambil dari seluruh Malaysia. Ekstrak DNA diambil dari 39 sampel ini dengan menggunakan kaedah pengekstrakan CTAB dan penanda inter simple sequence repeat (ISSR) digunakan untuk menganggarkan variasi gen. Hanya 7 penanda ISSR dipilih daripada 25 primer ISSR yang diuji. Kriteria pemilihan adalah berdasarkan kebolehan primer untuk menggandakan serpihan DNA yang kemudiannya mudah di skor pada gel agarose dan kebolehannya untuk menggandakan serpihan DNA yang sama. Secara keseluruhannya, sebanyak 70 lokus dikenalpasti dan 59 didapati polimorfik dengan purata 8.43 lokus polimorfik per penanda. Nilai Shannon yang digunakan untuk mengganggar variasi gen *C.citratus* dikira dengan menggunakan program POPGENE dan nilai tertinggi yang diperoleh adalah 0.2263 dimana ianya menunjukkan variasi gen yang rendah. Jarak genetik juga dikira dengan menggunakan program GenAlEx dan secara puratanya jarak genetik untuk semua sampel ialah 16.35. Berdasarkan data yang diperolehi, pokok filogenetik telah dibina dengan menggunakan kaedah UPGMA. Tiga kelompok dihasilkan, dimana 2 kelompok berkongsi dahan yang sama dan kelompok ketiga berada di dahan yang kedua.

ACKNOWLEDGEMENT

This project could not be accomplished without the help form many people. A special thanks to my supervisor Dr. Noor Baity Saidi, who had to read and corrected my thesis many times and this thanks also goes to Dr. Ng Wei Lun who had assist me during the experiment and help me doing the analysis of the data. Not to forget my previous supervisor, Prof. Tan Soon Guan who had gave me a chance to do this project. Here, I would like to express my gratitude to my project partner Syazana who had helped me a lot to do this project. This thanks also goes to the staff in faculty of Biotechnology and Molecular Sciences, staff in Animal and tissue culture lab who offered guidance and support. Lastly thanks to my family and friends who always provide me with support and love throughout this project.

Approval

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of degree of Science. The member of Supervisory Committee was as follows:

Dr. Noor Baity Binti Saidi, PhD
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

…….....……

(Prof Madya Dr. Janna Ong Binti Abdullah)
Head of Department Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences.
Universiti Putra Malaysia
Date:

Declaration

Declaration by undergraduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by the Department of Cell and Molecular Biology.
- written permission must be obtained from supervisor before thesis is published (in the form written, printed, or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials.
- there is no plagiarism or data falsification/ fabrication in the thesis. The thesis has undergone plagiarism detection software (TURNITIN).

Signature : …… Date:…….

Name and Matric No: ……

Declaration by Supervisor

This is confirmed that:

- the research conducted and the writing of this thesis was under supervision

Signature: ……..

Name of Chairman :……..

Supervisory committee

LIST OF TABLES

TABLES		PAGE
Table 3.1	Sampling sites	13
Table 3.4	A list of markers used	18
Table 4.1	The average of measurement taken from 15 samples of *C.citratus*	21
Table 4.2	Primer-wise score of PCR amplification in *C.citratus*	25
Table 4.3	Genetic diversity parameters among 7 ISSR markers for all 39 samples of *C.citratus*	27

LIST OF FIGURES

FIGURES		PAGE
Figure 2.2	An illustration of how ISSR is amplified	11
Figure 4.1	*Cymbopogoncitratus*	21
Figure 4.2	Polymorphic banding pattern of UBC 834 and UBC 841 among 39 genotypes.	24
Figure 4.4	Dendogram generated using UPGMA method by utilizing genetic distance data.	28

LIST OF ABBREVIATIONS

ISSR	Inter Simple Sequence Repeat
RFLP	Random fragment Length Polymorphism
AFLP	Amplified Fragment Length Polymorphism
RAPD	Random amplified Polymorphism DNA
SSR	Simple Sequence Repeat
PIC	Polymorphism Information Index
FFAs	free fatty acids
PCR	Polymerase Chain Reaction
DNA	Deoxyribose nucleic acids
CTAB	Cetyltrimethylammonium bromide
TE buffer	Tris EDTA Buffer
RNase	Ribonuclease
TBE	Tris Borate EDTA
UV	Ultraviolet
PVP	polyvinylpyrrolidone
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

1.0 INTRODUCTION

Genetic variation or also known as genetic diversity refers to the variation in DNA sequences between and within populations of organism. In plants, genetic variation depends on its evolution and the breeding system, ecological and geographical factors, past bottlenecks and human factors. The study of genetic variation in plants is important as it can give a better understanding on its evolution, increase knowledge on the taxonomy and its origin, and crucial for the conservation of the plants (Rao, V. R., 2002).

Cymbopogon is derived from Greek words ‘’Kymbe” (boat) and “pogon” (beard) referring to Flower spike arrangement (Shah *et al.* 2011). Under the genus of *Cymbopogon* (Family: Poaceae) there are approximately 140 species which widely distributed in semi-temperate to tropical regions of Asia. Based on studies done, 31 species are distributed throughout Asia (Khanuja *et al.*, 2005). *Cymbopogon* are stuffed perennial C_4 grasses with numerous stiff stems arising from short, rhizomatous rootstock (Shah *et al.*, 2011). *Cymbopogon* species that produce volatile oil such as palmarosa oil, lemongrass oil, citronella oil and ginger grass (rusa oil) are called as aromatic grasses (Rao, 1997). In Malaysia there are 2 species of *Cymbopogon*, they are *Cymbopogon citratus* (serai makan) or commercially known as lemongrass and *Cymbopogon nardus* (serai wangi).

In Malaysia *Cymbopogon nardus* are often used in lotion, soap and as insect repellent, it is not edible to be eaten. *C. citratus* is widely used in cooking and tea to give fragrance to the food. In traditional medication, *C.*

citratus able heal or reduces flatulence, irregular bowel movement, gastric irritability and to arrest vomiting. In addition, its extract is believed to be a good mosquito repellent (MP167 2012). *C. citratus* can be eaten and source of medication to human, therefore it is important to know the level of genetic variation in this plant. There are many uses of *C. citratus*, but the exploration of genetic variation in *C. citratus* could discover more benefits in it. The problem is, genetic variation of *C. citratus* is still unknown in Malaysia.

The past studies of *Cymbopogon* species were focused on phenotypic expression, herbage yield, the essential oil and the genetic differences on its oil constituent (Khanuja 2005; Karunamoorthi and Ilango, 2010). The most similar study with this project was written by Adhikari *et* at (2013) which using Random Amplified Polymorphic DNA (RAPD) analysis to assess the genetic diversity of indian elite clones of *Cymbopogon* species. Due to many significant reason of studying genetic variation, and proved by many paper that studied on *Cymbopogon* species, thus it is important also to know the genetic variation in Malaysian *C. citratus* which has not yet being discovered. The hypotheses of this project suggest that the genetic variation in Malaysian *C. citratus* maybe low, but the differences exist among the population.

Among of many types of the markers used; Random fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP)

and Random amplified Polymorphism DNA (RAPD), Inter Simple Sequence Repeat (ISSR) has proven to be the most efficient markers. ISSR markers are highly sensitive to detect genomic polymorphism and offer a great potential to determine intra and interspecific variation and this primers able to detect a unique nucleotide sequence patterns of small DNA fragments (400-800bp) (Reddy *et al*., 2002)). The main advantage of using ISSR is that no sequence data for primer construction are needed. Thus due to these reasons, ISSR was chosen as the marker in assessing genetic diversity in *C. citratus.*

The objective of this project is to primarily genotype the *C. citratus* that grows in Malaysia and to identify useful ISSR markers in *C. citratus.*

6.0 REFERENCES

Abegaz, B., Yohannes, P. G., & Dieter, R. K. (1983). Constituents of the essential oil of ethiopian cymbopogon citratus stapf. *Journal of Natural Products*, *46*(3), 424-426.

Adhikari, S., Saha, S., Bandyopadhyay, T. K., & Ghosh, P. (2015). Efficiency of ISSR marker for characterization of cymbopogon germplasms and their suitability in molecular barcoding. *Plant Systematics and Evolution*, *301*(1), 439-450.

Alam, M. A., Juraimi, A. S., Rafii, M. Y., Hamid, A. A., Arolu, I. W., & Latif, M. A. (2015). Genetic diversity analysis among collected purslane (portulaca oleracea L.) accessions using ISSR markers. *Comptes Rendus Biologies*, *338*(1), 1-11.

Hebert, P. D., & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic Biology*, *54*(5), 852-859. doi:P642568W06G713U8 [pii]

Karunamoorthi, K., & Ilango, K. (2010). Larvicidal activity of cymbopogon citratus. *European Review for Medical and Pharmacological Sciences*, *14*, 57-62.

Doyle, J. (1991). DNA protocols for plants. *Molecular techniques in taxonomy* (pp. 283-293) Springer.

Quisumbing, E. (1951). Medicinal plants of the philippines. *Tech.Bull.Dept.Agric.Phil.is.Philippine Medicinal Plants Drug Plants Medicinal Plants Materia Medica Pharmacognosy, Geog, Review Article General Article (PMBD, 185205153)*

Khanuja, S. P. S., Shasany, A. K., Pawar, A., Lal, R. K., Darokar, M. P., Naqvi, A. A., Kumar, S. (2005). Essential oil constituents and RAPD markers to establish species relationship in cymbopogon spreng. (poaceae). *Biochemical Systematics and Ecology, 33*(2), 171-186. doi:http://ezproxy.upm.edu.my:2099/10.1016/j.bse.2004.06.011

Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America, 102*(23), 8369-8374. doi:0503123102 [pii]

Kumar, J., Verma, V., Goyal, A., Shahi, A., Sparoo, R., Sangwan, R., & Qazi, G. (2009). Genetic diversity analysis in cymbopogon species using DNA markers. *Plant Omics J, 2*(1), 20-29.

MP167, M. P. (2012). Mosquito repellence of astrolochii hepii, cymbopogon citratus and ocimum gratissimum extracts and mixtures. *Bull.Environ.Pharmacol.Life Sci.;* 60-64.

Rao, V. R., & Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture, 68*(1), 1-19.

Ratnaparkhe, M., Santra, D., Tullu, A., & Muehlbauer, F. (1998). Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. *Theoretical and Applied Genetics, 96*(3-4), 348-353.

Reddy, M. P., Sarla, N., & Siddiq, E. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica, 128*(1), 9-17.

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of cymbopogon citratus, stapf (lemon grass). *Journal of Advanced Pharmaceutical Technology & Research, 2*(1), 3-8. doi:10.4103/2231-4040.79796 [doi]

Silva, Cristiane de Bona da, Guterres, S. S., Weisheimer, V., & Schapoval, E. E. (2008). Antifungal activity of the lemongrass oil and citral against candida spp. *Brazilian Journal of Infectious Diseases, 12*(1), 63-66.

Singh, S., Singh, H., Singh, A., & Tiwari, R. (2012). Genetic variability and character association in lemongrass (cymbopogon flexuosus L. stapf). *Journal of Spices and Aromatic Crops, 14*(2)

Wuthi-udomlert, M., Chotipatoomwan, P., Panyadee, S., & Gritsanapan, W. (2011). Inhibitory effect of formulated lemongrass shampoo on malassezia furfur: A yeast associated with dandruff. *Southeast Asian Journal of Tropical Medicineand Public Health, 42*(2), 363.

Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics, 20*(2), 176-183. doi:http://ezproxy.upm.edu.my:2099/10.1006/geno.1994.1151