



UNIVERSITI PUTRA MALAYSIA

**EVALUATION OF GENETIC VARIATION BETWEEN
cymbopogon citratus AND *C.nardus* USING ISSR AND DNA
SEQUENCING**

NUR SYAZANA MOHAMED ABU BAKAR

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AND *C.nardus* USING ISSR AND DNA SEQUENCING**

By

NUR SYAZANA BINTI MOHAMED ABU BAKAR

Thesis submitted to the Department of Cell & Molecular Biology, Faculty of
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Chair: Prof. Madya Dr. Janna Ong Abdullah, PhD

Faculty: Faculty of Biotechnology & Biomolecular Sciences

Cymbopogon citratus (*C. citratus*) and *Cymbopogon citratus* (*C. nardus*) are two aromatic grass species which can be found in Malaysia. *C. citratus* or commonly known as lemongrass is edible and has been widely used in Malay culinary dishes while *C. nardus* or citronella grass cannot be ingested and only used topically as an ointment. In this study, *C. citratus* and *C. nardus* samples collected around Peninsular Malaysia were characterized using inter-simple sequence repeats (ISSRs) to observe the genetic variation between both species. 28 ISSR primers were screened and 8 primers were selected based on reproducibility and ability to produce scorable bands. Out of 70 loci scored, 63 were found to be polymorphic. Genetic distance was calculated from the scored bands and UPGMA dendrogram was constructed. Three DNA samples from each species were sent for sequencing and a marker was developed based on single nucleotide polymorphism between *C. citratus* and *C. nardus*.

Abstrak thesis yang dikemukakan kepada Jabatan Biologi Sel & Molekul sebagai memenuhi keperluan untuk ijazah Biologi Sel & Molekul

**PENILAIAN VARIASI GENETIK ANTARA *Cymbopogon citratus* DAN *C.nardus*
MENGUNAKAN ISSR DAN PENJUJUKAN DNA**

Oleh

NUR SYAZANA BINTI MOHAMED ABU BAKAR

Jun 2015

Pengerusi: Prof. Madya Dr. Janna Ong Abdullah, PhD

Fakulti: Fakulti Bioteknologi & Biomolekular Sains

Cymbopogon citratus (*C. Citratus*) dan *Cymbopogon nardus* (*C. nardus*) adalah dua spesies rumput aromatik yang boleh didapati di Malaysia. *C.citratus* atau lebih dikenali sebagai serai makan boleh dimakan dan telah digunakan secara meluas dalam masakan-masakan Melayu manakala *C.nardus* atau serai wangi tidak boleh dimakan dan hanya disapu secara luaran sebagai ubat. Dalam kajian ini, sampel *C.citratus* dan *C.nardus* dikumpulkan di sekitar Semenanjung Malaysia dicirikan menggunakan inter simple sequence repeats(ISSRs) untuk melihat variasi genetik di antara kedua-dua spesies. 28 primer ISSR telah diuji dan 8 primer dipilih berdasarkan kebolehulangan dan keupayaan untuk menghasilkan band. Daripada 70 lokus, 63 didapati polimorfik dengan peratus sebanyak 90%. Jarak genetik telah dikira dari lokus dan UPGMA dendrogram telah dibina. Tiga sampel dari setiap spesies juga telah dihantar untuk penjujukan DNA dan

penanda dibangunkan berdasarkan perbezaan nukleotida tunggal antara *C.citratus* dan *C.nardus*.



Approval

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

Prof. Madya Dr. Janna Ong Abdullah, PhD

Associate Professor

Faculty of Biotechnology & Biomolecular Sciences

Universiti Putra Malaysia

Prof. Madya Dr. Janna Ong Abdullah, PhD

Head of Department Cell & Molecular Biology

Faculty of Biotechnology & Biomolecular Sciences

Universiti Putra Malaysia

Date:

Declaration

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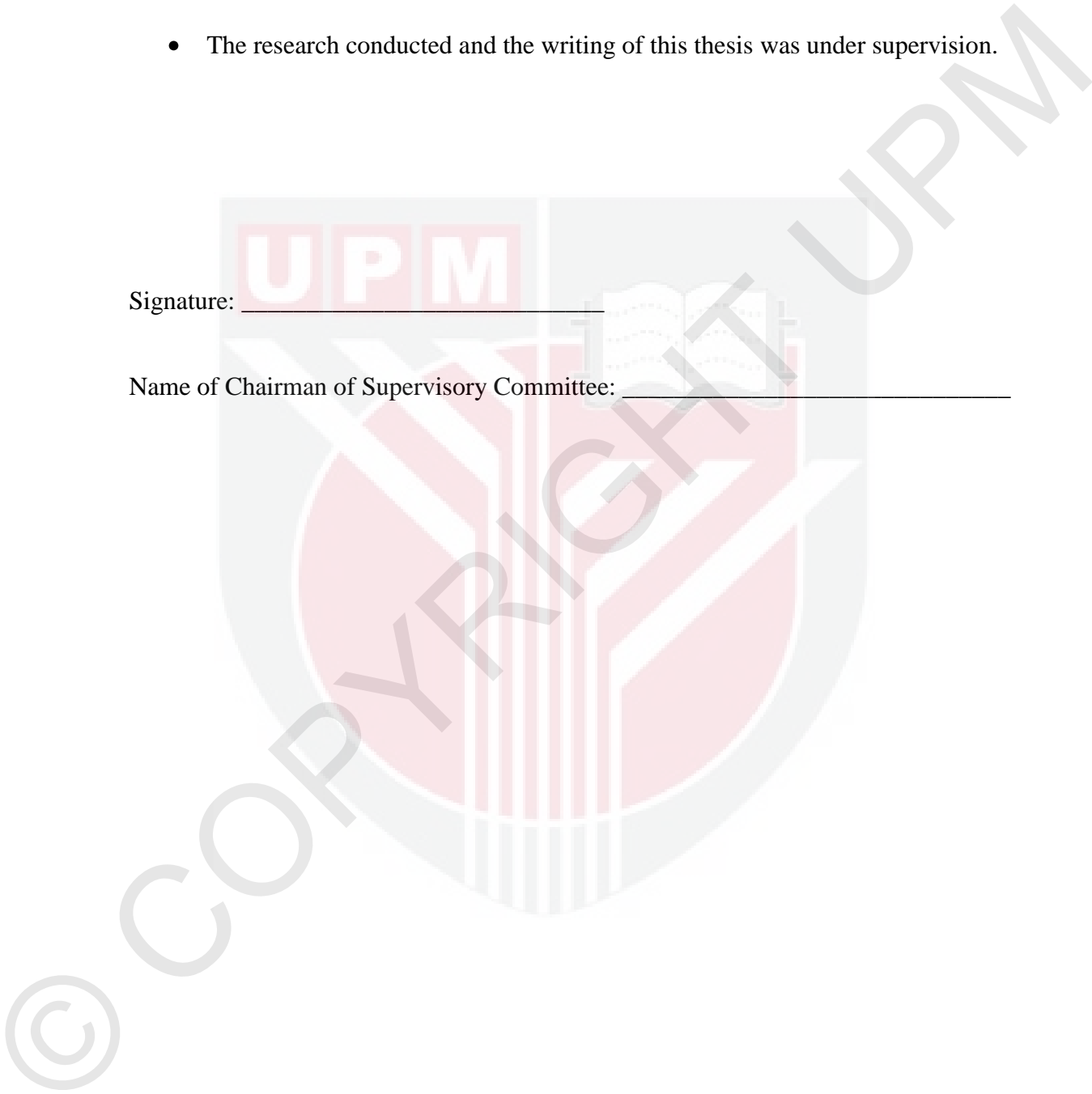
Declaration by Supervisor

This is to confirm that:

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

Signature: _____

Name of Chairman of Supervisory Committee: _____



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LIST OF ABBREVIATIONS

SNP : single nucleotide polymorphism

CTAB : Cetyltrimethylammonium bromide

PCR : Polymerase chain reaction

DNA : Deoxyribonucleic acid

UPGMA : Unweighted Pair Group Method with Arithmetic Mean

CHAPTER ONE

INTRODUCTION

Cymbopogon genus is a member of the family Poaceae which is known for their high essential oil contents. The essential oils are usually used for cosmetics, pharmaceuticals and perfumery applications. Cymbopogon are widely distributed across all continents where they are used for various purposes. In Malaysia, there are two commonly found species of Cymbopogon, *C. citratus* and *C. nardus*. *C. citratus* is widely used in variety of dishes as it is edible. However, *C. nardus* is inedible and has been used as traditional medicine and insect repellent.

Genetic variation is a naturally occurring genetic difference between individuals of the same species or other species. This variation permits flexibility and survival of a population when facing environmental changes. Consequently, genetic variation is often considered an advantage, as it is a form of preparation for the unexpected, such as preventing an infectious disease to attack and wipe out whole population. The assessment of genetic variation patterns in plant population has made many contributions in evolutionary biology, conservation genetics, plants breeding and ecological genetics. There are a few genetic variations studies on several species of Cymbopogon from India (Kumar et al, 2009). So far, none of Cymbopogon species in Malaysia has been studied for their genetic variations. The similar morphology of two commonly found *C. citratus* and *C. nardus* has made it difficult for people to identify

them. Thus, by using the marker developed in this study, identification of either one of the species was easier to be carried out.

Traditionally, biological variation has been estimated through the morphological characteristics of the plant. However, this method is inaccurate and unreliable because phenotypic traits are highly influenced by environmental conditions (Ruan *et al.*, 2009). The marker that will be used in this study is the Inter Simple Sequence Repeat (ISSR) or also known as Random Amplified Microsatellites (RAMs). This method is PCR-based where the primers contain microsatellite sequences and also degenerate anchors at 5' end. This method does not require genome sequence information; it leads to multilocus, highly polymorphic patterns and is a dominant marker (Mishra *et al.*, 2003). Through DNA sequencing, the variation between *C. citratus* and *C. nardus* can be observed at nucleotide level. Thus, a marker can be developed based on the identified variation and this will make it easier to differentiate *C. citratus* from *C. nardus*.

In this study, the genetic variation between *C. citratus* and *C. nardus* in Malaysia was detected using Inter Simple Sequence Repeat (ISSR) markers and DNA sequencing. There are altogether three objectives of this study. The first objective of this study is to identify genetic variation between *C. citratus* and *C. nardus* in Malaysia, the second objective is to develop a marker to differentiate *C. citratus* and *C. nardus* based on their sequence differences and the last one is to build a phylogenetic tree to show the relationship between the two species. Samples of *C. citratus* and *C. nardus* were collected around Malaysia and DNA was extracted from each of the samples using

CTAB method. Then, 28 ISSR primers were screened and the primers that produce clear and reproducible bands were used in subsequent step which was ISSR genotyping. Data analysis was done by scoring the bands manually, using GenAIEx ver. 6.5 (Peakall and Smouse, 2012) to calculate the genetic distance and MEGA 6 (Tamura et al. 2013) to construct UPGMA tree. Lastly, three samples from each species was sent for sequencing and two markers to differentiate *C. citratus* and *C. nardus*; trnLF.ForCn and trnLF.RevCn was developed based on single nucleotide polymorphism (SNP).

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