UNIVERSITI PUTRA MALAYSIA

PHENOTYPE AND GENOTYPE VARIATION, STEM CUTTING GROWTH PERFORMANCE AND POD MATURITY OF MORINGA OLEIFERA

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PHENOTYPE AND GENOTYPE VARIATION, STEM CUTTING GROWTH PERFORMANCE AND POD MATURITY OF *MORINGA OLEIFERA*

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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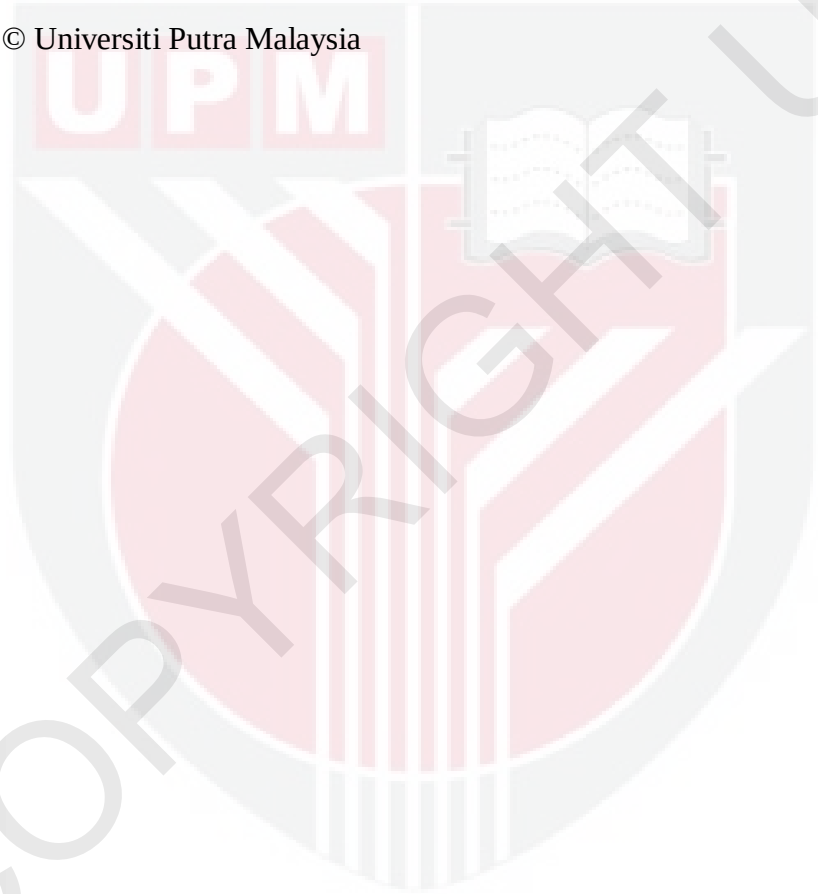

DEDICATION

This thesis dedicated to my loving husband Mohamed Hasrizal Hashim and my son Muhammad Iman Zhafran, my mother Fauziah, my father Mohamad, my mother in law Habibunishah and my father in law Hashim for their endless and boundless love, support, encouragement, and most of all for their ever continuous do'a for my life.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

DETERMINATION OF PHENOTYPE AND GENOTYPE AND STEM CUTTING GROWTH PERFORMANCE AND POD MATURITY OF *MORINGA OLEIFERA*

By

MUNIRAH MOHAMAD

September 2013

Chairperson: Siti Hajar Binti Ahmad, PhD
Faculty: Agriculture

Moringa oleifera is a multipurpose tree as it is cultivated for vegetable, spice, cosmestic oil and medicinal plant. *M. oleifera* oil contains all the main fatty acid including oleic acid which is very stable when used for frying. Moreover, it has the potential to become a new source of high oleic acid oil. Previous study only focused on medicinal and nutritional aspects of the tree parts. However, there is lack of information on the morphological and genetic variations of *M. oleifera* grown in Malaysia. Thus, the objectives of the study were to determine the phenotypic and genotypic variations among *M. oleifera* accessions, the performance of stem cuttings of two selected accessions as propagation material, and the growth and maturation of pod of a selected accession.

Morphological characteristics and ISSR molecular markers were used to assess levels of polymorphism across 20 accessions of *M. oleifera in situ* and *ex situ.* There were variations on morphological characteristics between accessions of *M. oleifera* based on stem girth, leaf length, leaf width, pod length, pod diameter, pod weight, seed number/pod, seed diameter and seed weight indicating the presence of genetic variability among accessions. Among the 20 accessions S05 had the highest seed weight followed by S04, with a difference of 3.7% seed weight. As for seed number/pod, accession P05 had the highest value followed by P03, with a difference of 4.7%. These two characteristics are beneficial in the selection of *M. oleifera* as they contribute to the total yield. The molecular analysis by using ISSR showed high polymorphism and abundance of ISSR sequences in *M. oleifera.* There were six cluster in the dendrogram with the majority of accessions from Perak and Selangor states being clustered separately.

In the experiment on the performance of stem cuttings, two accessions (S05 and P05) were selected as propagation materials based on the highest seed weight (S05) and seed number/pod (P05). Six types of cuttings, fresh and dried hardwood, fresh and dried semi-hardwood and fresh and dried softwood were evaluated as the most suitable as planting materials. *M. oleifera* could be propagated successfully by using fresh semi-hardwood cutting compared to the other five types of cuttings. Cuttings from fresh semi-hardwood had better shoot and adventitious root production, thus had a good overall growth performance.

For the determination of pod growth and maturation, accession S05 was selected to be grown in the field plot. In this experiment, pod and seed characteristics were determined 5-9 days after anthesis (DAA). Pod size increased gradually at 9 DAA, followed by a rapid increment at 9-37 DAA. Pods were observed to be at edible stage at 35-37 DAA. Pod size increased slowly between 37 to 44 DAA as pods were observed to become fully matured (oil extraction stage). Then, the increase in pod size levelled off at 44 DAA. Colour of edible pods and fully matured pods were observed to be light green and brown, respectively.

Morphological characteristics and ISSR molecular markers showed high polymorphism and grouped to the accessions in six clusters. Subsequently, for selection of propagation materials, cuttings for fresh semi-hardwood showed optimum growth performance compared to other materials. For growth and development of seeds and pods, full maturity was achived at 44 DAA. These findings could be used as background information for breeding and improvement program of *M. oleifera* in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENENTUAN FENOTIPIK DAN GENOTIPIK DAN PRESTASI PERTUMBUHAN KERATAN BATANG SERTA KEMATANGAN LENGAI *MORINGA OLEIFERA*

Oleh

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Pengerusi: Siti Hajar Binti Ahmad, PhD
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Moringa oleifera adalah pokok pelbagai guna ditanam sebagai sayur-sayuran, rempah, memasak dan alat kosmetik dan juga sebagai tanaman perubatan. *M. oleifera* mengandungi semua asid lemak utama termasuk asid oleik yang sangat stabil walaupun diguanakan untuk menggoreng. Tambahan lagi, ia mempunyai potensi untuk menjadi satu sumber baru minyak tinggi asid oleik. Kajian sebelum ini hanya memberi tumpuan kepada aspek-aspek perubatan dan pemakanan satu bahagian pokok;Walau bagaimanapun, tiada penyelidikan yang telah dilakukan mengenai variasi morfologi dan genetik *M. oleifera* di Malaysia. Oleh itu, objektif kajian ini adalah untuk menentukan variasi fenotip dan genotip antara aksesi *M. oleifera*, prestasi keratan batang sebagai bahan biakbaka untuk dua aksesi terpilih dan pertumbuhan dan kematangan lengai bagi aksesi yang dipilih.

Ciri-ciri morfologi dan penanda ISSR telah digunakan untuk menilai tahap kepelbagaian terhadap 20 aksesi *M. oleifera*. Terdapat variasi pada ciri-ciri morfologi antara aksesi *M. oleifera* berdasarkan lilitan batang, panjang daun, lebar daun, panjang lengai, diameter lengai, berat lengai, bilangan biji benih/lengai, diameter biji benih dan berat biji benih menunjukkan kehadiran kepelbagaian genetik di kalangan aksesi. Antara 20 aksesi, S05 mempunyai berat biji benih tertinggi diikuti oleh S04, dengan perbezaan berat biji benih sebanyak 3.7%. Bagi bilangan biji benih/lengai, aksesi P05 mempunyai nilai tertinggi diikuti oleh aksesi P03, dengan perbezaan sebanyak 4.7%. Kedua-dua ciri-ciri ini sangat berguna dalam kriteria pemilihan *M. oleifera* kerana cirri-ciri tersebut menyumbang kepada hasil keseluruhan. Analisis molekul dengan menggunakan ISSR menunjukkan kepelbagaian yang tinggi dan bilangan jujukan ISSR yang banyak dalam *M. oleifera*. Terdapat enam kelompok dalam dendrogram dengan majoriti aksesi dari negeri Perak dan Selangor dikelompokkan secara berasingan.

Bagi prestasi keratan batang, dua aksesi (S05 dan P05) telah dipilih sebagai bahan pembiakan berdasarkan berat benih tertinggi (S05) dan bilangan biji benih/lengai (P05) paling banyak. Enam jenis keratan iaitu kayu keras segar dan kering, kayu separa keras segar dan kering serta kayu lembut segar dan kering telah dinilai bagi menentukan bahan tanaman paling sesuai. *M. oleifera* didapati boleh dibiakkan dengan jayanya dengan menggunakan keratan kayu separa keras segar berbanding lima jenis keratan lain. Keratan daripada kayu separa keras segar menghasilkan pucuk dan akar yang lebih baik, sekali gus mempunyai prestasi pertumbuhan keseluruhan yang baik.

Bagi penentuan pertumbuhan dan kematangan lengai, aksesi S05 telah dipilih untuk ditanam di ladang. Dalam eksperimen ini, ciri-ciri lengai dan biji benih telah ditentukan pada 5 hingga 9 hari selepas berputik (DAA). Saiz buah meningkat secara beransur-ansur pada 9 DAA, diikuti dengan kenaikan secara mendadak pada 9 hingga 37 DAA. Lengai diperhatikan untuk berada pada peringkat yang boleh dimakan pada 35 hingga 37 DAA. Saiz buah meningkat perlahan-lahan antara 37 hingga 44 DAA apabila buah diperhatikan menjadi matang sepenuhnya (peringkat minyak bole diekstrakkan). Kemudian, peningkatan dalam saiz lengai terhenti pada 44 DAA. Warna buah yang boleh dimakan didapati adalah hijau muda dan matang sepenuhnya adalah coklat telah diperhatikan.

Akhirnya, kajian ini memberikan maklumat yang berharga mengenai ciri-ciri fenotip dan genotip aksesi bagi *M. oleifera* dan penemuan ini boleh digunakan sebagai maklumat latar belakang untuk program pembiakan dan peningkatan spesis ini di Malaysia.

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My deepest gratitude goes to my beloved husband Hasrizal Hashim and all family members for their help and continuous moral support throughout my study.
I certify that a Thesis Examination Committee has met on September 2012 to conduct the final examination of Munirah Mohamad on his thesis entitled "Determination of Morphological Characteristics and Genetic Variation among *Moringa oleifera* Accessions" in accordance with the Universities and Univesity Colleges Act 1971 and the Constitution of the Pertanian Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

APPROVAL

I certify that a Thesis Examination Committee has met on 29th May 2014 to conduct the final examination of Md. Muklesur Rahman on his thesis entitled "Vase Life Enhancement of Mokara Red Orchid with *Jatropha curcas* L., *Psidium guajava* L. and *Andrographis paniculata* (Burm.f.) Wall. Ex. Nees Leaf Extracts" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

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DECLARATION

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I hereby confirm that:

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Supervisory Committee: Hasanah Mohd Ghazali, PhD

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LIST OF ABBREVIATIONS

σ^2	Variance
^oC	degree Celcius
AFLP	amplified fragment length polymorphism
AMOVA	analysis of molecular variance
ANOVA	analysis of variance
bp	base pairs
cm	Centimetre
C.V.	coefficient of variation
DNA	deoxyribonucleic acid
dNTP	2'-deoxynucleoside 5'- triphosphate
FAO	Food and Agriculture Organization
GA	genetic advance
GCV	genotypic coefficient of variation
g	Gram
G_{ST}	coefficient of gene differentiation
GV	genotypic variance
h	Nei's (1973) gene diversity
H_S	gene diversity within populations
H_T	total gene diversity
I	Shannon's information index
IPGRI	International Plant Genetic Resources Institute
ISSR	inter simple sequence repeat
masl	metre above sea level
MS	mean squares
µg	Microgram
µl	Microlitre
µM	Micromolar
mM	Milimolar
n_a	observed number of alleles
n_e	effective number of alleles
N	North
Nm	estimate of gene flow
NPB	number of polymorphism bands
NTSYS	numerical taxonomy multivariate analysis system
PC	principal component
PCA	principal component analysis
PCR	polymerase chain reaction
PCV	phenotypic coefficient of variation
PPB	percent of polymorphic bands
ppm	parts per million
r	correlation coefficient
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism

rpm	revolution per minute
s	second
S.E.	standard error
SSR	simple sequence repeat
S.D.	standard deviation
Taq	*Thermus aquaticus*
TE	tris ethylenediaminetetraacetic acid buffer
UPGMA	unweighted pair group method using arithmetic averages
UV	Ultraviolet
V	Volt

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CHAPTER 1

INTRODUCTION

Moringa oleifera seed oil, known as the Ben oil, is widely used in the watch industry. The oil is also pleasant tasting and edible (Lowell, 1999). In Haiti, the oil has been used as general culinary and salad oil (Price, 1985). The oil contains all the main fatty acids found in olive oil, and therefore, could be a possible substitute to the olive oil after some modifications (Abdulkarim *et al*., 2004).

M. oleifera has potential to be one of the sources of fat and oil as there is a need to find a supplement for the existing ones. The extracted oil from *M. oleifera* seeds contains high unsaturated fatty acids such as oleic, palmitic, stearic, arachidic and behenic acids. There are many reasons for the current push in high-oleic acid oils. Corbett (2003) in a recent issue of INFORM highlighted the benefits of using such oils. It is generally accepted as healthier oil which is rich in monounsaturated fatty acids (e.g. oleic acid) that is more stable to oxidative rancidity and stable as deep frying oils. One of the multipurpose trees that have been cultivated in many countries is *M. oleifera* which has the potential to become a new source of oil for Malaysia.

Previous research has shown that by studying the morphological characteristics of *M. oleifera*, we can distinguish between species and varieties. Olson (2002) showed that differing species groups within the Moringa genus have been proposed based on the characters studied including the leaf and floral morphology. Polymorphism and highly heritable morphological characteristics were some of the earliest genetic markers employed in scientific investigation, and may still be optimal for certain plant germplasm management application, particularly in *M. oleifera*. At the same time, molecular markers have recently played an important role in identifying variation and estimating genetic diversity.

Thus, *M. oleifera* has the potential to be one of the world's most useful plants for oil production. In Malaysia, the *M. oleifera* tree is not grown widely. One or two trees may be planted around the house compounds and the pods and shoots are used in cooking. Since, *M. oleifera* is so important, an intensive conservation has to be carried out and the germplasm must be characterized to enhance utilization. Studies on morphological characteristics and genetic variation among accessions of *M. oleifera* available in Malaysia are still lacking. These studies are needed to determine the morphological and genetic characteristics among available accessions of *M. oleifera.*

The different cuttings, either hardwood, semi-hardwood or softwood differ in rooting ability during propagation. According to Kantarli (1993), the highest rooting was

obtained with cuttings taken from the middle part of shoots as sown in *Shorea selanica*. Cuttings from the basal portion (hardwood) of shoots produce poor rooting ability and high rates of mortality because of lignifications. Similarly, cuttings from the uppermost (softwood) of the shoots also produce lower rooting ability. Normally people cuttings were used as propagation method which were usually dried the before being propagated. Howerver, there is lack of information on root and shoot initiation during propagation from the different stem cuttings of *M. oleifera*. Such information is necessary for producing planting materials needed for commercial field planting of the crop.

M. oleifera seeds are dark brown, globular and about 1cm in diameter, with three whitish papery wings (Ramachandran *et al.*, 1980). The mature seed pods remain on the tree for several months before splitting open and releasing the seeds which are dispersed by wind, water and probably animals (Parotta, 1993). Mature pods contain ripe seeds that are used for planting to germinate the next crop (Palada and Chang, 2003). For oil extraction, the seed in the pods are allowed to dry and turn brown on the tree (Palada and Chang, 2003). Thus, it is important to observe every single day after anthesis to determine the exact day for the pods to dry and turn brown (fully mature). Besides that, it is important to know the relationship between flower production, pod formation and seed set to get highest yield of oil among accessions.

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