UNIVERSITI PUTRA MALAYSIA

GENETIC VARIABILITY AND SELECTION OF HIGH ROOT YIELD AND HIGH EURYCOMANONE CONTENT GENOTYPES IN MALAYSIAN TONGKAT ALI (Eurycoma longifolia Jack) GERMPLASM.

SENTOOR KUMERAN GOVINDASAMY

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By

SENTOOR KUMERAN GOVINDASAMY

Thesis Submitted to the School of Graduate Studies,

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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SENTOOR KUMERAN GOVINDASAMY

October 2014

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Faculty: **Agriculture**

Eurycoma longifolia or better known as tongkat ali in Malaysia is a herb used mainly as general tonic, aphrodisiac and antipyretics. It belongs to the Simaroubaceae family and found mainly in lowland forest of Malaysia thriving under forest canopy. Currently, in Malaysia, the major supply of tongkat ali comes from wild collections of forest reserves. Over harvesting of wild tongkat ali cause extinction of the species. There were not many recorded information on the study of tongkat ali in the aspects of breeding, agronomic, pest and disease management, post harvest technology and bio-molecular assay in Malaysia. Thus, it is important to identify accessions of tongkat ali which exhibit high growth rate and good root yield in the germplasm collection for farmers use and cultivation. This will significantly reduce wild collections and increase cultivation of tongkat ali for industrial use. Among various parts of tongkat ali, the tap root of the plant appears to be in use greatly. In this study, 10 accessions of tongkat ali were collected from the forest of Peninsular Malaysia for evaluation of viability in non shade growing condition, growth and yield performance, chemical content analysis, genetic similarity, G × E interactions and heritability of traits. The accessions were planted in RCBD experimental design in three replicates at two locations, namely MARDI Serdang and MARDI Kluang. The height and girth data were collected at six month intervals. Beside, other morphological data such as internode length, leaf length, number of leaflets per leaf, leaflet area, leaflet width and length were taken during the final collection of

height and girth data. Other parameters taken into consideration include yield data of total root yield, lateral and tap root yield and plant crown weight. In order to investigate on the DNA extraction of tongkat ali, leaflets samples were collected simultaneously during experimentation. Data collected were analyzed statistically using SAS procedures for Analysis of variance (ANOVA) and the New Duncan Multiple Range Test for progeny impact between treatments and locations. Molecular markers data were analyzed using NTSYS-pc 2.1 to generate genetic similarity cluster dendrogram.

From the data collected on plant height and girth in Serdang and Kluang, significant differences among the accessions in terms of growth were observed. Among the accessions, the highest mean plant height obtained of MEL 10 was 355 cm in Serdang and 395 cm in Kluang. MEL 10 in Serdang and Kluang produced the biggest girth among the treatments with girth size of 68 mm and 72 mm respectively. Significant differences were obtained among the number of leaflets per leaf, leaf length and leaflet area in Serdang and number of leaflets, leaflet area, leaf length, leaflet width and tree crown weight in Kluang. Meanwhile, fresh root yield of tongkat ali in both locations exhibited significant variations among the 10 accessions of tongkat ali. MEL 08 in Serdang and Kluang showed highest root yield of tongkat ali. The analysis of G × E showed differences in genotypes performance in the two locations in term of height and girth size. Cluster analysis at 0.75 mean average distances among the accessions indicated the existence of four distinctive types of tongkat ali accessions among the collected 10 accessions using plant morphological characters analysis. Accession MEL 12 had the highest concentration of eurycomanone in the roots. Chemical content was a highly heritable trait as the broad-sense heritability values were above 70% in terms of eurycomanone content in tap root, lateral root and leaves. An IMP molecular marker assisted cluster analysis showed at 0.75 coefficient value, six distinctive clusters of tongkat ali among the 10 collected tongkat ali accessions.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KEPELBAGAIAN GENETIK DAN PEMILIHAN GENOTIP HASIL AKAR DAN KANDUNGAN EURIKOMANON YANG TINGGI DARI JANAPLASMA TONGKAT ALI (*Eurycoma longifolia* Jack) DI MALAYSIA

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Eurycoma longifolia atau lebih dikenali sebagai tongkat ali di Malaysia adalah herba yang digunakan secara meluas sebagai tonik umum, agen afrosidia dan antipiretik. Tumbuhan ini tergolong dalam famili Simaroubaceae dan dijumpai terutamanya di kawasan hutan tanah pamah serta hidup di bawah naungan kanopi pokok hutan. Pada masa kini, bekalan utama untuk tongkat ali adalah dari pengumpulan hasil hutan yang tumbuh secara liar. Penuaian berlebihan dan tidak terkawal tongkat ali liar akan menyebabkan kepupusan spesies ini. Tidak ada banyak maklumat dilaporkan mengenai kajian tongkat ali dari segi pembiakbakaan, agronomi, kawalan perosak dan penyakit, teknologi lepas tuai dan cerakin bio-molekular. Oleh itu adalah penting untuk mengenalpasti aksesi-aksesi tongkat ali yang menunjukkan kadar pertumbuhan dan hasil tuaian akar yang tinggi di dalam janaplasma yang dikumpulkan untuk kegunaan penanaman komersial. Ini akan secara siknifikan mengurangkan pengumpulan tongkat ali liar dan meningkatkan penanaman untuk kegunaan industri. Di antara pelbagai bahagian pokok tongkat ali, akar tunjangnya merupakan bahagian yang paling banyak digunakan. Dalam kajian ini, 10 aksesi tongkat ali yang dikumpulkan daripada hutan semenanjung Malaysia telah dinilai untuk viabiliti di kawasan terbuka tanpa pokok lindungan bagi penilaian prestasi pertumbuhan, hasil, dan kandungan kimia. Sepuluh aksesi ini telah ditanam mengunakan rekabentuk blok penuh rawak (RCBD) dengan tiga replikasi di dua lokasi iaitu MARDI Serdang dan MARDI Kluang. Data prestasi ketinggian dan ukur lilit batang telah dikumpulkan pada setiap selangan enam bulan. Selain dari itu, data morfologi seperti panjang ruas antara daun, panjang daun, bilangan anak daun per daun, luas anak daun, panjang serta lebar anak daun telah diambil pada akhir

ekperimen. Ciri lain yang telah dikumpulkan ialah data hasil akar keseluruhan, hasil akar tunjang dan sisi serta berat kanopi pokok. Bagi kajian molekular, DNA telah diekstrak dari sampel anak daun pokok tongkat ali dari plot kajian di MARDI Serdang. Data yang dikumpulkan telah dianalisa dengan menggunakan perisian SAS untuk analisa varians (ANOVA) dan ujian berganda Duncan baru (DNMRT). Data penanda molekular telah dianalisa dengan menggunakan perisian NTSYS-pc2.1 bagi mendapatkan carta dendrogram kesamaan genetik.

Keputusan kajian ini menunjukkan terdapat perbezaan antara aksesi bagi ciri ketinggian dan ukurlilit pokok di Serdang dan Kluang. Aksesi MEL 10 memberikan ketinggian pokok yang paling tinggi iaitu 355 cm di Serdang dan 395 cm di Kluang. Aksesi MEL 10 juga memberikan pokok yang mempunyai ukurlilit batang yang paling besar di Serdang dan Kluang (68 mm dan 72 mm, masing-masing). Perbezaan yang bererti telah dicerap dikalangan aksesi untuk ciri bilangan anak daun per daun, panjang daun dan luas anak daun di Serdang serta bilangan anak daun per daun, luas anak daun, panjang daun, lebar anak daun dan berat kanopi di Kluang. Selain dari itu, hasil akar tongkat ali basah di dua lokasi menunjukkan variasi yang bererti di antara 10 aksesi tongkat ali tersebut. Aksesi MEL 08 di Serdang dan Kluang menunjukkan hasil akar yang tertinggi. Analisis G×E menunjukkan perbezaan dari segi kadar pertumbuhan genotip untuk pertambahan ketinggian dan ukurlilit di dua lokasi tesebut. Analisa kluster kesamaan genotip pada purata beza tahap 0.75 menunjukkan terdapat 4 kluster dikalangan 10 aksesi tongkat ali berdasarkan ciri morfologi pokok. Aksesi MEL 12 mempunyai kandungan eurikomanon yang paling tinggi dalam akarnya. Kandungan kimia, eurikomanon merupakan ciri yang sangat tinggi keupayaan untuk diperturunkan kepada generasi seterusnya, iaitu memberikan nilai heritabiliti melebihi 70% bagi eurikomanon dalam akar tunjang, akar sisi dan daun. Kajian bantuan penanda molekular IMP pada nilai pekali 0.75, mengklusterkan 10 aksesi tongkat ali yang telah dikumpulkan kepada enam kluster.

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APPROVAL

I certify that an Examination Committee has met on to conduct the final examination of Sentoor Kumeran Govindasamy on his M.Sc. thesis entitled "**GENETIC VARIABILITY AND SELECTIONS OF HIGH ROOT YIELD AND HIGH EURYCOMANONE CONTENT GENOTYPES IN MALAYSIAN TONGKAT ALI (*Eurycoma Longifolia* Jack) GERMPLASM**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the M. Sc.

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TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENT		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xv
LIST OF APPENDICES		xvi
LIST OF ABBREVIATIONS		xvii
LIST OF SYMBOLS		xix
CHAPTER		
1.	**INTRODUCTION**	1
2.	**LITERATURE REVIEW**	
	2.1. *Eurycoma longifolia* Jack	4
	2.2. Utilization of tongkat ali	4
	2.3. Secondary metabolits of tongkat ali	7
	2.4. Genotype by environment interaction	8
	2.5. Statistical analysis of G × E interaction	10
	2.6. Analysis of variance	10
	2.7. Extraction of eurycomanone in Tongkat Ali	11
	2.8. Plant molecular genotyping	11
3.	**GENETIC VARIABILITY ON MORPHOLOGICAL AND ROOT YIELD TRAITS of *Eurycoma longifolia* GERMPLASM IN MALAYSIA**	
	3.1. Introduction	14
	3.2. Materials and Methods	16
	3.2.1. Collection and nursery establishment of tongkat ali accessions	16
	3.2.2. Experimental site and design	18
	3.2.3. Land preparation	18
	3.2.4. Agronomic practices	19
	3.2.5. Duration of field experiment	20
	3.2.6. Data collection	20
	3.2.7. Statistical analysis	23
	3.3. Results	25
	3.3.1. Plant height and girth measurement Serdang site	25
	3.3.2. Plant morphological characters measurement	

in Serdang 31
3.3.3. Plant height and girth measurement in Kluang site 35
3.3.4. Plant morphology characters measurement in Kluang site 43
3.3.5. Root yield measurement in Serdang site 45
3.3.6. Root yield measurement in Kluang site 48
3.3.7. Comparison of growth performance between Serdang and Kluang sites 51
3.3.8. Comparison of plant morphological characters between Serdang and Kluang sites 58
3.3.9. PCV, GCV and heritability estimates for tongkat ali progenies in Serdang 62
3.3.10. Correlation analysis of tongkat ali morphological and yield characters 64
3.3.11. Cluster analysis of ten tongkat ali accessions 66
3.4. Discussion 68
3.5. Conclusion 72

4. EVALUATION AND SELECTION OF ACCESSIONS WITH HIGH EURYCOMANONE CONTENT FROM *Eurycoma longifolia* GERMPLASM IN SERDANG.
4.1. Introduction 73
4.2. Materials and Methods 74
4.2.1. Sampel plants and experimental design 74
4.2.2. Harvesting and extraction procedure of samples 74
4.2.3. Statistical analysis 75
4.3. Results 76
4.3.1. ANOVA and means comparison of eurycomanone content in 10 tongkat ali accessions 76
4.3.2. Analysis of estimation of heritability 79 4.3.3. Correlation analysis 80
4.4. Discussion 82
4.5.Conclusion 88

5. APPLICATION OF INTER MINIATURE TERMINAL ELEMENT (MITE) POLYMERASE (IMP) MARKERS TO TONGKAT ALI ACCESSIONS FOR GENETIC DIVERSITY STUDY
5.1 Introduction
5.2 Material and Methods 89
5.2.1. DNA extraction and analysis 91
5.3 Results 92
5.4 Discussion 97

5.5 Conclusion 98

6. **GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH** 99

REFERENCES 101

APPENDICES 111

BIODATA OF STUDENT 115

LIST OF PUBLICATION 116

LIST OF TABLES

Table		Page
3.1	Collecting locations of tongkat ali accessions.	17
3.2	Geographical conditions of experimental plots.	18
3.3	Fertilizer application schedule.	19
3.4	Estimation of mean square and variance component	23
3.5	Estimation of variance components of combined ANOVA	24
3.6	Mean square values for plant height at 6 months interval in Serdang	26
3.7	Mean value of plant height of 10 accessions of tongkat ali grown in Serdang at 6 months intervals	27
3.8	Mean Square values for plant girth size in Serdang	29
3.9	Mean value of plant girth of 10 accessions of tongkat ali grown in Serdang at 6 months intervals	30
3.10	Mean square values for seven morphological characters of 10 tongkat ali accessions	32
3.11	Mean values for plant morphological characters in 10 accessions of tongkat ali grown in Serdang.	33
3.12	Mean Square values for plant height in Kluang.	36

3.13	Mean value of plant height of 10 accessions of tongkat ali grown in Kluang at 6 months intervals	37
3.14	Mean Square values for plant girth size in Kluang.	40
3.15	Mean value of plant girth of 10 accessions of tongkat ali grown in Kluang at 6 months intervals	41
3.16	Mean Square for different plant morphological characters in Kluang.	44
3.17	Mean values of plant morphological characters in 10 accessions of tongkat ali grown in Kluang.	44
3.18	Mean square values of root yields in Serdang.	47
3.19	Mean values for root yield among 10 accessions of tongkat ali in Serdang.	47
3.20	Mean Square value for root yields in Kluang.	49
3.21	Means of root yield of among 10 accessions of tongkat ali in Kluang	49
3.22	Mean square values for plant height of tongkat ali in two locations.	52
3.23	Mean square values for plant girth of tongkat ali in two locations	53
3.24	Means of plant height among 10 tongkat ali accessions in two locations of Serdang and Kluang.	54
3.25	Means of plant girth among 10 tongkat ali accessions in two locations of Serdang and Kluang.	54
3.26	Mean Square values of G × E for root yield in two locations.	56
3.27	Means of root yield among 10 accessions of tongkat ali in two locations of Serdang and Kluang.	57
3.28	Mean Square values for plant morphology characters in two locations.	59
3.29	Means of 10 accessions of tongkat ali morphology characters in two locations of Serdang and Kluang.	61

3.30	Heritability analysis of different plant characters in Serdang.	63
3.31	Correlation analysis of tongkat ali morphological and yield characters.	65
4.3.1	Means square of eurycomanone yield (mg/5 g sample).	77
4.3.2	Eurycomanone content in various plant parts of different accessions of *Eurycoma longifolia*.	78
4.3.3	Variance components analysis on tongkat ali eurycomanone content.	79
4.3.4	Correlation between eurycomanone yield and plant morphological characters.	81

LIST OF FIGURES

Figure		Page
3.1	Dendrogram of cluster result of 10 tongkat ali genotypes using eighteen morphological characters	67
5.1	IMP4_700 PCR amplification of 10 accessions of tongkat ali.	93
5.2	IMP7_700 PCR amplification of 10 accessions of tongkat ali.	94
5.3	IMP12_700 PCR amplification of 10 accessions of tongkat ali.	95
5.4	Cluster analysis of 10 accessions of tongkat ali in Serdang experimental plot based on genetic similarity coefficient computed by NTSYS-PC software from CMDV Serdang.	96

LIST OF APPENDICES

	Page
Appendix A1. Experimental Plot And RCBD Arrangement Of Tongkat Ali Treatments Open Planting In Serdang And Kluang.	111
Appendix A2. Tongkat Ali Treatment And Guard Row Plants Plan.	112
Appendix A3. Soil Mineralogical, Physical Condition And Chemical Characteristics Of Experimental Plot	113
Appendix A4. Tongkat Ali Plot In Serdang And MEL 10 Accessions Sample Plants At 12 MAP	114

LIST OF ABBREVIATIONS

%	Percentage
µg	Microgram
AGV	Agro-ecology
ANOVA	Analysis of variance
cm	Centimeter
CV	Coefficient of variation (%)
DF	Degree of freedom
E	Environment
E(TR)	Eurycomanon tap root content
E(LR)	Eurycomanon lateral root content
E(LF)	Eurycomanon leaf content
E(TRR)	Eurycomanon total root content
E(TP)	Eurycomanon total plant content
FAO	Food and Agriculture Organization
g	Gram
GCV	Genotypic coefficient of variation
G×E	Genotype by environment
kg	Kilogram
LA	Leaflet area
LL	Leaflet length
LW	Leaflet width
m	Meter
MARDI	Malaysian Agricultural Research & Development Institute
MEL	Malaysian *Eurycoma Longifolia*
mm	Milimeter
MS	Mean square
MT	Metric ton
NLPR	No of leaflets per Leaf
PCV	Phenotypic coefficient of variation
RL	Leaf Length
SD	Standard deviation

SE	Standard error
SS	Sum of squares

LIST OF SYMBOLS

a_i	The means of the i^{th} genotype over all environments (linear regression)
b_i	The regression coefficient (slope) of the i^{th} genotype (linear regression)
c	The dummy index for counting components
e	The number of environment
g	The number of genotype
h	A dummy index for counting environmental explanatory variables
i	A dummy index for counting genotypes
j	A dummy index for counting environment
m	The number of dependent variables
n	The number of cases
p	The probability value
r	The rank of a matrix
r	The number of blocks (replicates)

s	The number of independent variables
E	Expectation
I_j	The j^{th} environment index
σ^2	The variance component of the random effect
μ	Grand mean
β_j	The additive main effect of the j^{th} environment
˚C	Celcius
$Block_{k(j)}$	The k^{th} block effect within the j^{th} environment

CHAPTER 1

INTRODUCTION

Medicinal and aromatic plants or commonly known as herbs were the earliest source of drugs and medicine to human being and animals. The use of herbs for healing dates back as far as prehistoric times and has been woven into the culture and civilization of people. Plants are a rich source of secondary metabolites that have medicinal and aromatic properties. Herbs are utilized for human usage in the form of drugs, antioxidants, flavors, fragrances, dyes, insecticides and pheromones. The use of synthetic drugs led to a decline in the use of herbal compounds, so that at one time it was believed by many that the synthetic drugs would perhaps completely replace the use of traditional plant-derived medicines (Indu Bala and Ng, 2004). Most synthetic drugs were obtained from herbs itself in the form of isolated derivatives and it is estimated that at least 100,000 such secondary metabolites are now known to occur in 50,000 plant species and 4,000 new secondary metabolites are being discovered every year from a variety of plant species.

In recent years, a new trend emerged in the medicinal world whereby herbal medicine has gained its place in modern medicine. Herbal medicine has been found to have some interesting secondary compounds which cannot be copied by western medicine. Some of the modern medicines have a lot of side effects, which cannot be found in traditional medicines. Given these important credentials, many professionals including world leading health authorities have accepted traditional medicine as more holistic in its approach in curing ailments (Indu Bala and Ng, 2004). Consequently, the current global herbal drug market has reached a level of US $62 billion, which is expected to grow to US $5 trillion by the year 2050 (*Joshi et al*., 2004). The world market for herbal medicines including herbal products and raw materials is actually growing at an annual rate of 5–15%. The rapid increase in traditional medicine industry worldwide is also reflected in Malaysia where there is an increase demand for medicinal plant by Malaysian consumers. Statistics show that the current herbal industry in Malaysia is estimated at RM2 billion in the year 2013.

Tongkat ali or its scientific name *Eurycoma longifolia* inhabits the lowland forest of Malaysia, Thailand and Indonesia (Picture 1). Locally this plant is found in both lowland and highland forest and widely collected for its medicinal and commercial values. Tongkat ali is also referred as ‘Malaysian ginseng’. This plant is traditionally used as a general tonic, after childbirth tonic, aphrodisiac, antidotal, antihypertensive, antipyretic and febrifuge (Indu Bala and Ng, 2004). The main part of the tree used by traditional medicine practitioners is the roots but in the commercial industry the stem and leaves are also being used for its

medicinal values (Noh *et al*., 2004). Treelet in nature, tongkat ali grows up to 10 m in height. Often a mature tree has a diameter of 10-13 cm. Tongkat ali have an odd-pinnate compound type of leaves and the leaflets are arranged in opposite pairs.

Picture 1: Tongkat ali or *Eurycoma longifolia* plant at one year age

The tree usually has oblong leaf shape but in certain collected samples, the leaves are lanceolate in shape. The leaf color is usually green to a dark green. Tongkat ali trees have no branches and in rare cases, there maybe 2-3 branches in a tree. Flowers of tongkat ali have five corolla and red in color. The flowers are small and odor-less. Flowers are situated compactly on branched panicles which arise from leaf axils. Tongkat ali has small sized fruits which is red or brown in color and has a diameter of 2 to 2.5 cm. Currently there is only one method of propagation for tongkat ali which is by seeds. Tongkat ali cannot be propagated by stem cuttings or leaf cuttings. Tongkat ali plants can yield good root weight in a period of 4-5 years. The tap root is considered to have more medicinal value compared to adventitious roots. Tongkat ali have no real pest infestation on its plants but *Atteva scrodoxa*, a type of caterpillar do cause considerable damage during early stage of open field establishment.

Commercial uses of tongkat ali are in beverages and pharmaceutical industries. In the beverage industry, premixture of coffee and tongkat ali is one of the popular and highly in demand beverage by locals. Dried tongkat ali roots are also sold as teas and capsules. The woody stem is also used to produce wooden cup which is used to store water and the water is believed to contain medicinal

benefits. The chemical constituents in tongkat ali which have been reported for health benefits are eurycomanol, eurycomanone and eurycomalactone (Rafidah *et al*., 2004). Many studies of tongkat ali were done in the field of extraction of its chemical compounds, identification of its compound and usage of the metabolites in many industries such as nutraceutical, medicine, pharmacology, food and beverages industries. There were not many recorded information on the study of tongkat ali in the aspects of breeding, agronomic, pest and disease management, post harvest technology and bio-molecular assay in Malaysia. This was due to the long period of plant maturity before harvesting which made this type of study highly costly and time consuming. Another reason for the unavailability of up-stream information on tongkat ali was due to the industry's major source of tongkat ali was from wild collections and not from agricultural output. The usage of wild collections will destroy the natural tongkat ali population of this country. Eventually the wild collections which hold the full genotype information of tongkat ali will perish and leads to genotypic extinction of tongkat ali. Thus in this study several objectives that can boost the knowledge of tongkat ali growth, yield, G x E interactions, diversity and chemical content studies were done.

The objectives of this study were:

1. To assess the viability of establishing tongkat ali field planting under non shade condition.
2. To analyze genetic diversity and genotype-environment interaction of tongkat ali germplasm in Malaysia.
3. To identify tongkat ali accessions which exhibit superior growth rate, high eurycomanone content in leaves and roots, and high root yield for commercial production.
4. To analyze genetic diversity among tongkat ali accessions using IMP markers.

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